VOLUME 38 MAY 1960 NUMBER 3

# *Canadian Journal of Botany*

*Editor:* D. L. BAILEY

*Published by* THE NATIONAL RESEARCH COUNCIL
OTTAWA CANADA

# *Canadian Journal of Botany*

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Canadian Journal of Botany

*Issued by* THE NATIONAL RESEARCH COUNCIL OF CANADA

VOLUME 38 MAY 1960 NUMBER 3

# SOME WEED HOSTS OF PRATYLENCHUS PENETRANS IN PREMIER STRAWBERRY PLANTATIONS[1]

J. L. TOWNSHEND AND T. R. DAVIDSON

## Abstract

Fifty-five species of weeds and seven of cultivated plants were collected from root rot areas in strawberry plantations and found to be infected with *Pratylenchus penetrans* (Cobb 1917) Filip. & Stek. 1941. Perennial plant species with soft-textured roots contained larger populations of *P. penetrans* and had more extensive root necrosis than those plant species with hard-textured roots.

## Introduction

*Pratylenchus penetrans* (Cobb 1917) Filip. & Stek. 1941 has been found associated with a wide variety of plants. The relationship of this parasite to the stunting of tomato plants was determined by Mountain and Fisher (10). Also, Mountain and Koch (11) found that *P. penetrans* would attack tobacco. Various workers have reported on the association of this nematode with root troubles in strawberry (2, 4, 6, 15). This parasite is also associated with the peach replant failure in some parts of Ontario (8, 9) and with cherry decline in New York State (7). In 1953 Jensen (5) demonstrated that *P. penetrans* will invade many gramineous and leguminous crops. The susceptibility of 164 cultivated plants to injury by *P. penetrans* was tested by Oostenbrink (12). The hosts included woody perennials, floral plants, gramineous and vegetable crops, and a single weed, *Taraxacum officinale* Weber. Mountain and Fisher (10) are the only other workers to have reported finding *P. penetrans* in the roots of weeds, but they did not name the infected species. During the investigation of the role of *P. penetrans* in strawberry root rot, the first author found dandelion to be a host of this nematode. The present study was undertaken to determine the potential importance of weeds that grow in strawberry plantations in Southern Ontario as reservoirs of nematode inoculum.

[1]Manuscript received December 23, 1959.
Contribution from the Plant Pathology Laboratory, Research Branch, Canada Department of Agriculture, St. Catharines, Ontario.



[The previous number of Can. J. Botany (38, 93–266) was issued March 15, 1960.]

## Methods

In 1958 weeds were collected from four Premier strawberry plantations in the Niagara peninsula known to be infested with *Pratylenchus penetrans*. Collections were made also from a fifth infested field that had been in Premier, clean cultivated for a year, and in July planted to buckwheat.

Weeds were collected every 2 weeks from April to August. Specimens were taken from at least five root rot areas in each plantation, as *P. penetrans* could be found more consistently and in greater numbers in these areas. In fields where strawberries had been ploughed down the weeds were collected at random.

Gray's Manual of Botany (3) and Bailey's Manual of Cultivated Plants (1) were used to identify the plants. Doubtful identifications were verified or corrected by the Division of Botany, Canada Department of Agriculture, Ottawa.

The roots of most plant species were examined for lesions, the characteristics of which were noted. These lesions were dissected to determine the occurrence of *P. penetrans*.

*Pratylenchus penetrans* was extracted from the roots by Young's technique (16). For each plant species to be tested, portions of root were excised at random from at least five specimens and placed in a pint sealer. A solution containing 0.0004% ethoxyethyl mercury chloride and 0.1% streptomycin sulphate was used in the extraction to suppress bacteria and fungi. The roots were incubated for 4 weeks at 65° F. All counts were calculated as the number of *P. penetrans* per gram of dried root.

The texture of the roots of each plant species was determined by gross examination, by dissection of lesions, and by the degree of breakdown of the roots during the extraction process.

Five hundred and fifty *P. penetrans* from the roots of 45 species of weeds were killed and relaxed in hot water, fixed in TAF (2% triethanolamine and 7% formalin), mounted on slides in lactophenol, and examined microscopically as to species.

## Results

*Identification of Nematodes*

The nematodes were identified as *Pratylenchus penetrans* (Cobb 1917) Filip. & Stek. 1941. Males were noted in all collections. Wide variations in the shape of the female tail were noted, some of these variations being similar to those illustrated by Taylor (13). The presence of striations around the female tail-tip of some specimens suggested that they might be *P. pratensis* (de Man 1880) but they differed from those illustrated by Thorne (14). The striations of a typical female *P. pratensis* are at right angles to the perimeter of the tail whereas such striations in these specimens were at acute angles. Furthermore the presence of a spermatheca in the female suggests that these forms were aberrant *P. penetrans*. No other species of *Pratylenchus* was found in these mounts.

*Hosts of Pratylenchus penetrans*

*Pratylenchus penetrans* was found in the roots of 55 weed and seven cultivated plant species belonging to 52 genera in 23 families (Table I). Sixty-three per cent of the weeds in which *P. penetrans* was found belonged to the families Compositae and Cruciferae.

TABLE I

Some weed and cultivated hosts of *Pratylenchus penetrans*

| Host | No. specimens collected | Life span of host | Root texture | Av. no. *P. penetrans* per gram of dried root | Lesions |
|---|---|---|---|---|---|
| Equisetaceae | | | | | |
| *Equisetum arvense* L. (field horsetail) | 5 | P | H | 349 | None observed |
| Gramineae | | | | | |
| *Triticum aestivum* L. (wheat*) | 10 | A | S | 1,978 | Not examined |
| *Phleum pratense* L. (timothy*) | 5 | P | S | 11,556 | Not examined |
| Polygonaceae | | | | | |
| *Rumex crispus* L. (curled dock) | 60 | P | S | 3,742 | Rough root |
| *Polygonum aviculare* L. (knotweed) | 10 | A | H | 482 | Rough roots |
| *Polygonum persicaria* L. (lady's-thumb) | 25 | A | S | 1,542 | None observed |
| *Polygonum convolvulvus* L. (wild buckwheat) | 5 | A | H | 414 | None observed |
| *Fagopyrum esculentum* Moench (common buckwheat*) | 25 | A | H | 437 | None observed |
| Chenopodiaceae | | | | | |
| *Chenopodium album* L. (lamb's quarters) | 35 | A | H | 1,039 | None observed |
| Amaranthaceae | | | | | |
| *Amaranthus retroflexus* L. (red-root pigweed) | 20 | A | H | 427 | None observed |
| Portulacaceae | | | | | |
| *Portulaca oleracea* L. (common purslane) | 10 | A | H | 251 | None observed |
| Caryophyllaceae | | | | | |
| *Arenaria serpillifolia* L. (thyme-leaved sandwort) | 5 | A | H | 91 | Not examined |
| *Stellaria media* (L.) Cyrillo (common chickweed) | 60 | A or WA | S | 1,865 | None observed |
| *Cerastium vulgatum* L. (common mouse-ear chickweed) | 10 | P | S | 23,607 | None observed |
| Ranunculaceae | | | | | |
| *Ranunculus abortivus* L. (kidneyleaf-buttercup) | 5 | P | S | 7,727 | Dark brown to black |
| Cruciferae | | | | | |
| *Erysimum cheiranthoides* L. (wormseed mustard) | 10 | A or WA | H | 36 | None observed |
| *Rorippa sylvestris* (L.) Bess. (creeping yellow cress) | 5 | P | S | 27,680 | Not examined |
| *Brassica kaber* (DC.) L.C. Wheeler (wild mustard) | 15 | A | H | 800 | Not examined |

TABLE I (*Continued*)

Some weed and cultivated hosts of *Pratylenchus penetrans* (*Continued*)

| Host | No. specimens collected | Life span of host | Root texture | Av. no. *P. penetrans* per gram of dried root | Lesions |
|---|---|---|---|---|---|
| *Brassica oleracea* var. *capitata* L. (cabbage*) | 7 | B | H | 3,236 | None observed |
| *Barbarea vulgaris* R. Br. (yellow rocket) | 5 | B or P | S | 774 | None observed |
| *Sisymbrium altissimum* L. (tumbling mustard) | 10 | A or WA | H | 7 | None observed |
| *Sisymbrium loeselii* L. (tall hedge mustard) | 10 | A | H | 42 | None observed |
| *Capsella bursa-pastoris* (L.) Medic. (shepherd's purse) | 55 | A or WA | H | 1,387 | None observed |
| *Thlaspi arvense* L. (stinkweed) | 5 | A or WA | H | 125 | None observed |
| *Lepidium campestre* (L.) R. Br. (field peppergrass) | 5 | A or B | S | 6,947 | Not examined |
| Rosaceae | | | | | |
| *Potentilla norvegica* L. (rough cinquefoil) | 15 | A,B, or P | S | 3,844 | Rough roots |
| Leguminosae | | | | | |
| *Trifolium pratense* L. (red clover*) | 15 | B | S | 2,399 | Pale brown |
| *Trifolium hybridum* L. (alsike clover*) | 10 | P | S | 4,567 | Brown to black |
| *Melilotus alba* Desr. (white sweet clover*) | 5 | B or P | S | 9,655 | Not examined |
| *Medicago lupulina* L. (black medick) | 25 | A | S | 6,349 | Black |
| Balsaminaceae | | | | | |
| *Impatiens capensis* Meerb. (spotted touch-me-not) | 5 | A | H | 200 | None observed |
| Malvaceae | | | | | |
| *Malva neglecta* Wallr. (common mallow) | 15 | B | S | 1,212 | Brown |
| Guttiferae | | | | | |
| *Hypericum punctatum* Lam. (dotted St.-John's-wort) | 15 | P | H | 2,521 | Rough roots |
| Onagraceae | | | | | |
| *Epilobium* L. sp. (fireweed) | 5 | P | S | 2,954 | Orange |
| Umbelliferae | | | | | |
| *Daucus carota* L. (wild carrot) | 5 | A or B | S | 3,636 | Not examined |
| Asclepiadaceae | | | | | |
| *Asclepias syriaca* L. (common milkweed) | 15 | P | S | 782 | Black |
| Convolvulaceae | | | | | |
| *Convolvulus arvensis* L. (field-bindweed) | 5 | P | H | 248 | Yellow to brown |
| Labiatae | | | | | |
| *Lamium amplexicaule* L. (clasping henbit) | 10 | A or WA | S | 2,919 | None observed |

TABLE I (*Concluded*)

Some weed and cultivated hosts of *Pratylenchus penetrans* (*Concluded*)

| Host | No. specimens collected | Life span of host | Root texture | Av. no. *P. penetrans* per gram of dried root | Lesions |
|---|---|---|---|---|---|
| *Lamium hybridum* Vill. (hybrid henbit) | 15 | A | S | 582 | None observed |
| Solanaceae | | | | | |
| *Solanum dulcamara* L. (bittersweet) | 5 | P | H | 529 | Rough roots |
| *Solanum nigrum* L. (black nightshade) | 5 | P | H | 729 | Rough roots |
| Scrophulariaceae | | | | | |
| *Veronica arvensis* L. (corn-speedwell) | 15 | A | H | 108 | Pale brown |
| Plantaginaceae | | | | | |
| *Plantago major* L. (common plantain) | 30 | P | S | 4,358 | Dark brown |
| Compositae | | | | | |
| *Solidago* spp. (golden rod) | 30 | P | S | 1,304 | Orange to brown |
| *Erigeron annuus* (L.) Pers. (annual daisy-fleabane) | 20 | A or WA | S | 13,637 | Orange to brown |
| *Erigeron strigosus* Muhl. (white-top fleabane) | 5 | A | S | 2,325 | Not examined |
| *Erigeron canadensis* L. (Canada fleabane) | 5 | A | H | 712 | Rough root |
| *Gnaphalium uliginosum* L. (low cudweed) | 15 | A | H | 1,277 | Rough roots |
| *Ambrosia artemisiifolia* L. (common ragweed) | 15 | A | H | 291 | Brown |
| *Anthemsis cotula* L. (mayweed) | 15 | A | S | 6,804 | Red |
| *Matricaria matricarioides* (Less.) Porter (pineapple-weed) | 5 | A | S | 205 | Orange |
| *Chrysanthemum leucanthemum* L. (ox-eye-daisy) | 10 | P | H | 390 | Orange to brown |
| *Senecio vulgaris* L. (common groundsel) | 20 | P | S | 7,225 | Reddish brown |
| *Arctium minus* (Hill) Bernh. (common burdock) | 10 | B | S | 117 | Dark brown |
| *Cirsium arvense* (L.) Scop. (Canada thistle) | 5 | P | S | 4,572 | Brown |
| *Onopordum acanthium* L. (Scotch thistle) | 10 | B | S | 3,106 | Purple to reddish brown |
| *Cichorium intybus* L. (chicory) | 10 | P | S | 2,261 | Orange |
| *Taraxacum officinale* Weber (common dandelion) | 60 | P | S | 7,225 | Reddish brown |
| *Sonchus arvensis* L. (perennial sow-thistle) | 20 | P | S | 1,500 | Yellow to reddish brown |
| *Sonchus oleraceus* L. (annual sow-thistle) | 10 | A | S | 2,488 | Reddish orange |
| *Lactuca scariola* L. (prickly lettuce) | 20 | A or B | S | 8,408 | Yellow to brown |
| *Hieracium pratense* Tausch (yellow hawkweed) | 10 | P | S | 18,100 | Reddish purple |

NOTE: A, annual; WA, winter annual; B, biennial; P, perennial; S, soft-textured; H, hard-textured.
*Cultivated plants.

*Host–Parasite Relationships*

The roots of all weeds sampled contained *P. penetrans*. The average population per gram of dried root varied from 7 in tumbling mustard to 27,680 in creeping yellow cress. There was no apparent relationship between population density and the plant family since the Polygonaceae, Caryophyllaceae, Cruciferae, and Compositae each contained a group of weeds infested by large, and a second by small numbers of *P. penetrans*. However, three relationships became evident when the weeds were arbitrarily classified as (i) having hard- or soft-textured roots, (ii) annuals or perennials, and (iii) containing large or small numbers of nematodes. The division between large and small nematode populations was arbitrarily set at 1000 per gram, a value that the latter rarely approached.

First, weeds with soft-textured roots contained more nematodes than those with hard-textured roots ($\chi^2 = +30.239$; $P_{0.001} = 10.827$).[2] Second, the roots of perennial weeds usually contained larger populations of *P. penetrans* than those of annuals ($\chi^2 = +9.625$; $P_{0.01} = 6.635$). Third, the roots of most perennial weeds were soft-textured whereas those of annuals were hard ($\chi^2 = +13.138$; $P_{0.001} = 10.827$). The chi-square values suggest that the development of large populations in the roots of a weed host is more closely related to or dependent upon root texture than upon life span. Since most weed species with soft-textured roots are perennials, ideal conditions are provided for the development and survival of large populations of *P. penetrans*.

*Pratylenchus penetrans* was also recovered from the roots of 14 of the 15 species of weeds collected from the field that had been clean cultivated for a year and kept relatively free from weeds. The populations of *P. penetrans* in the roots of these weeds were comparable with those found in the same weeds in the four existing strawberry plantations.

*The Lesion*

Weeds with soft-textured roots had extensive root lesions and appeared to be more susceptible to root injury than weeds with hard-textured roots ($\chi^2 = +10.977$; $P_{0.001} = 10.827$). From these lesions adults, larvae, and eggs of *P. penetrans* could be readily dissected. Generally the lesions on the main and lateral roots were discrete, minute, and scratch-like. Some, however, were longitudinally extended in narrow stripes. When very numerous the lesions coalesced to involve a considerable area of the root. The color of the lesions appeared to vary with the host. Microscopically the lesions were slightly depressed often split longitudinally and occasionally transversely. The necrotic tissue lying centrally in the lesions frequently sloughed off to expose colonies of *P. penetrans*. The lesions appeared to be limited to the cortex, some being a few cells deep and others extended to, but did not appear to involve the stele. The stripe-like lesions, commonly a few cells wide, appeared to have developed by the migration of the nematode from the original lesion.

[2]The chi-square ($\chi^2$) values were calculated from 2×2 contingency tables testing the null hypothesis.

## Discussion

Oostenbrink (12) found 164 cultivated, and Jensen (5) 24 gramineous and leguminous plant species to be hosts of *Pratylenchus penetrans*. In the present paper 55 weed species are reported to be hosts of this parasite. The three papers comprise a host list of 232 plant species belonging to 126 genera in 51 families. *Pratylenchus penetrans* is obviously a polyphagous plant parasite. However, there is considerable variation in the ability of *P. penetrans* to infect and reproduce in these hosts. It appears that *P. penetrans* can penetrate and reproduce more readily in perennial weeds with soft-textured roots than in annual weeds with hard-textured roots. The former hosts could act as reservoirs for large numbers of overwintering *P. penetrans* posing a potential threat to any succeeding crop susceptible to this nematode. Other effective sources of nematode inoculum could include such hosts as chickweed which is a winter annual and can occur as a continuous succession of plants. Therefore weed control should be an important part of any nematode control program.

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# COCHLIOBOLUS SATIVUS

## III. EFFECT OF ULTRAVIOLET RADIATION[1]

R. D. TINLINE, J. F. STAUFFER, AND J. G. DICKSON


## Abstract

The lethal and mutagenic effect of ultraviolet light on two isolates of *Cochliobolus sativus* was studied. Conidia of a dark-spored strain were considerably more resistant to high doses of radiation than those of a white-spored strain. Ultraviolet radiation increased the frequency of mutation in the fungus. Mutants were recovered that differed from their parents in degree of sporulation, rate of growth, pigmentation, amount of aerial mycelium, topography and consistency of colony, and pathogenicity to wheat seedlings. Tetrads from crosses between weakly and highly pathogenic strains indicated that pathogenicity was not a clearly segregating character.


## Introduction

Apparently *Helminthosporium sativum* P. K. and B. (*Bipolaris sorokiniana* (Sacc. in Sorokin) Shoemaker (17)), an incitant of the common root-rot disease of wheat and barley, exhibits little parasitic specialization in its hosts (7). Strains of the fungus, however, differ in virulence and concomitantly, varieties of host plants differ in resistance (15). Differentiation into physiological races on the basis of parasitism therefore might be expected. Presumably this situation may be clarified by genetic studies. Although unreported from the field, the perfect stage, *Cochliobolus sativus* (Ito and Kurib.) Drechs. ex Dastur, is known in laboratory cultures (10, 16, 21) and offers a tool whereby such genetic studies may be conducted. The inheritance of some characters through the perithecial stage has been previously reported (22).

Strains of the fungus differing widely in pathogenicity and other characters appeared requisite for the investigation. Consequently, despite the high degree of variability observed in laboratory cultures of the organism (2, 9, 12, 13, 19) a program of induced mutation was initiated. The present paper describes the lethal and mutagenic effect of ultraviolet radiation on two strains of *C. sativus*.

## Materials and Methods

Two monoascosporic strains, A18, a dark-spored isolate of mating type *a*, and $K_3$, a white-spored isolate of mating type *A*, that were approximately equally pathogenic to wheat seedlings, were maintained on potato dextrose agar. No variants were observed in these strains cultured on this medium.

Aqueous spore suspensions were prepared from 10-day-old cultures. Conidiophores and other debris were removed by filtering several times through cheesecloth and finally by passing the conidia through a water column. The concentration was adjusted to 25,000–125,000 spores/ml by haemacytometer counts.

[1]Manuscript received December 29, 1959.
Joint contribution from Canada Department of Agriculture Research Station, Saskatoon, Sask. (No. 37), and the Departments of Botany and Plant Pathology, University of Wisconsin, Madison, Wisconsin.

The irradiation apparatus was similar to that used by Boone *et al.* (1), and consisted of a water-cooled, high-pressure, 1000-w General Electric AH-6 mercury vapor lamp, a Bausch and Lomb monochromator with quartz lenses, an irradiation vessel with quartz windows, and a thermopile. Sidearms on the irradiation vessel permitted continuous stirring and the removal of samples during treatment. Prior to use the vessel was sterilized with 70% ethyl alcohol followed by rinsing with sterile distilled water.

A wave length of 2750 Å was used throughout the study. The energy absorbed by the spores was calculated by the following formula:

$$\text{Ergs/spore} = \frac{T \times I_0 - \{(a/100)\, I_0\} - I_t + \{(b/100)\, I_t\} - \{(c/100)(I_0 - (a/100) I_0)\}}{N}$$

where $T$ = treatment time (sec); $I_0$ = incident energy (sensitivity of thermopile (ergs/sec cm) × galvanometer deflection (cm)); $I_t$ = transmitted energy; $a$ = per cent of incident energy lost at front window; $b$ = per cent of transmitted energy reflected by the rear window; $c$ = per cent of energy absorbed by the water; and $N$ = number of spores in suspension (plate count).

Samples taken during irradiation were suspended in sterile distilled water. Aliquots (1.0 ml) of the resulting suspension containing approximately 100 spores/ml were distributed on the surface of a synthetic (presumably minimal) agar medium (MM) in Petri dishes. Each plating was replicated 10 times. The medium was similar to that employed by Boone *et al.* for *Venturia inaequalis* (1) and had the following composition: (g/l.) $KNO_3$, 3.12; $K_2HPO_4$, 0.75; $KH_2PO_4$, 0.75; $MgSO_4.7H_2O$, 0.5; NaCl, 0.1; $CaCl_2.2H_2O$, 0.1; glucose, 5.0; agar, 20.0; (mg/l.) $ZnSO_4.7H_2O$, 0.396; $CuSO_4.5H_2O$, 0.079; $MnSO_4.4H_2O$, 0.0405; $MoO_3$, 0.0175; and $FeC_6H_5O_7.3H_2O$, 0.5355.

Colony counts and numbers of mutants were recorded 4 to 7 days and approximately 14 days after plating, respectively. Mutants were those colonies which wholly or partially differed visibly in any way from the usual type of colony. Hyphal tip or conidial isolations were made from the mutant sectors.

To ensure favorable conditions for growth on MM the pH and temperature optima of the isolates were determined in preliminary tests. The growth tube method of Ryan *et al.* (14) was employed. Tubes containing 10 ml of media were adjusted to desired pH levels by the addition of sterile HCl or NaOH after autoclaving. After transfer of conidia to one end, the tubes were incubated at various temperatures. The total increment in linear growth in 10 days was the basis of comparison.

On many agar media, including MM, *C. sativus* has a spreading growth habit that complicates the counting of colonies. Tatum *et al.* (20) reported that the sodium salt of desoxycholic acid, a constituent of oxgall, restricted the colony size of *Neurospora* and *Syncephalastrum*. Consequently oxgall (bile salts) was incorporated into MM in various concentrations to determine its paramorphogenic effect on *C. sativus*.

Numerous isolates from treated spores were screened for changes in pathogenicity to Marquis wheat seedlings. Seed was surface disinfected with a mercuric chloride solution (1:1000) (5 minutes), immersed in a sterile 2% solution of sodium sulphide (10 minutes), and washed in several changes of sterile distilled water to remove traces of mercury. Then it was placed on the scarred surface of cultures of the isolates on potato dextrose agar in test tubes, five seeds per tube. Relative pathogenicities were determined after 10 days' incubation at 24° C.

Progeny of crosses between mutants and monoascosporic isolates of the wild type obtained by the method of Tinline (22) were tested for differences in pathogenicity. Thatcher wheat seed, surface disinfected by the procedure described above, was placed on moist blotting paper in Petri dishes. The kernels were inoculated with a drop of aqueous spore suspension from an isolate culture and incubated at 24° for 4 days (18). Disease ratings were calculated from the seedling lesions by the application of McKinney's formula (11).

The slow-growing mutants were screened for nutritional deficiencies by comparing their growth in liquid MM, MM fortified with vitamins (thiamin hydrochloride, 100 $\mu$g; calcium pantothenate, 200 $\mu$g; nicotinic acid, 200 $\mu$g; riboflavin, 100 $\mu$g; pyridoxine hydrochloride, 200 $\mu$g; inositol, 5 mg; biotin, 5 $\mu$g; and vitamin B12, 2 $\mu$g; per liter of medium), MM supplemented with vitamin-free casein hydrolyzate (10 ml/l.), and MM with vitamins and casein hydrolyzate added. Serial transfer of the isolates on the media minimized a possible carry-over of growth factors with the conidial or hyphal inocula.

## Results

On MM the strains grew rapidly and sporulated uniformly. Isolate A18 developed over a pH range of 3 to 12 at 24°. Both isolates grew best at 24 to 28° C and at pH 8 but developed well at the temperature of 24° C and at pH 6 (Fig. 1), and the latter conditions were employed throughout the study.

Bile salts were relatively ineffective in inhibiting spore germination; for example, spore germination of isolate A18 was 100, 87, 91, 95, and 70% at salt concentrations of 0, 0.001, 0.01, 0.1, and 1.0% respectively, within a concentration range which effectively reduced the spreading habit of growth of colonies (Fig. 2). Consequently, to facilitate counts of survivors and mutants bile salts were added routinely to MM at a concentration of 0.05 to 0.1%.

Ultraviolet radiation exerted a considerable lethal effect on the spores of *C. sativus* (Fig. 3). Despite the significance of linear regressions of dose on survival, curves of better fit were obtained by the cubic polynomial $Y = a + bX + cX^2 + dX^3$ and such are presented. Although differences between tests with each isolate were apparent, the most pronounced differences were those between the two isolates. The conidia of the dark-spored strain were more resistant than those of the white-spored strain to high doses of radiation.

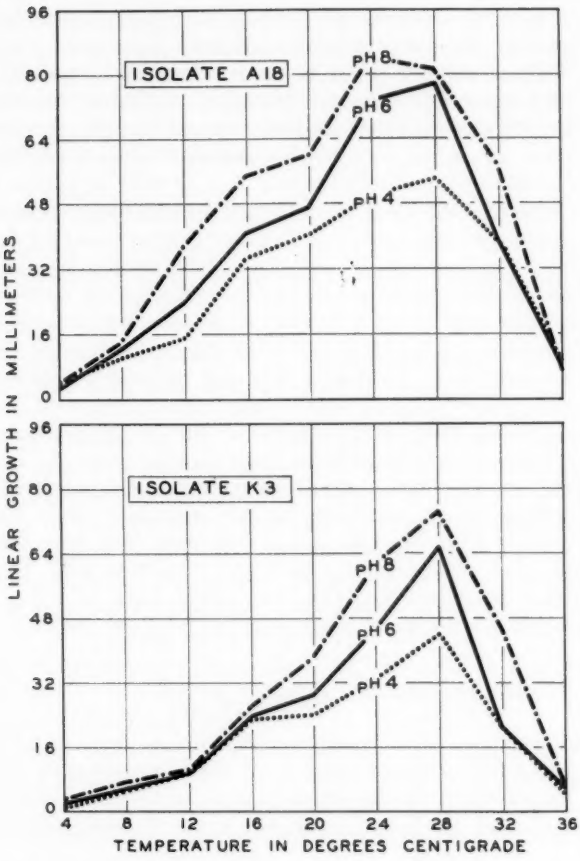


FIG. 1. Effect of temperature and pH on growth of isolates of *C. sativus* on a synthetic agar medium.

Ultraviolet radiation increased the frequency of mutation in both strains of *C. sativus*. The percentage of mutants among the survivors increased with time of treatment up to a maximum and then usually declined. The results, however, were extremely erratic and when plotted did not produce comparable curves. On MM the white-spored strain produced more visible mutants than the dark-spored strain regardless of treatment. Mutants differed from their parents in such characters as sporulation, rate of growth, pigmentation, amount of aerial mycelium, topography, and consistency of colony, among others (Fig. 4).

PLATE I

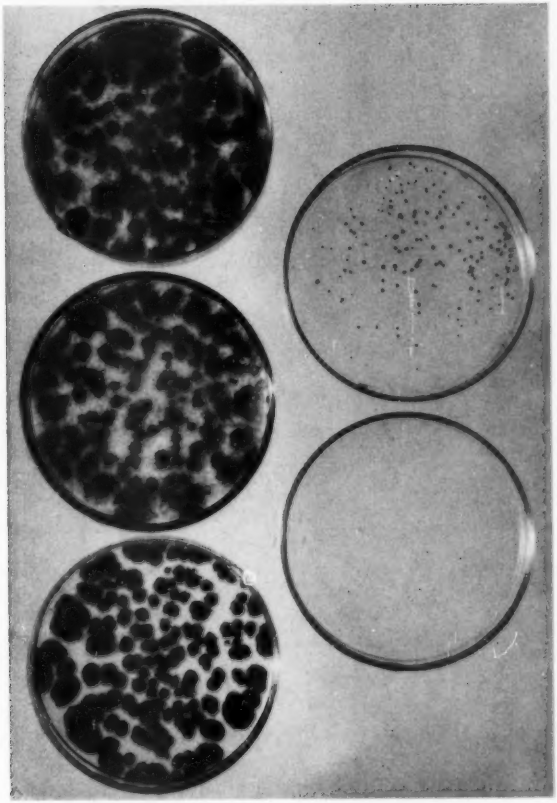

FIG. 2. Growth restriction in *C. sativus* by bile salts. From left, reading down, concentrations were 0, 0.001, 0.01, 0.1, and 1.0%, respectively.

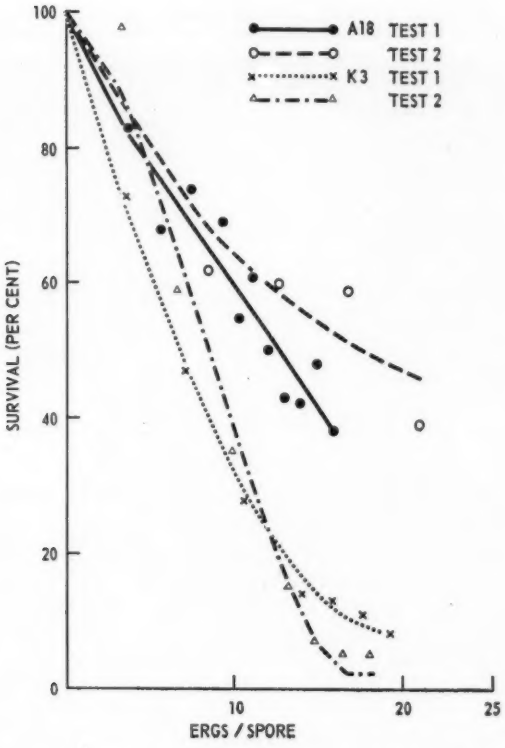


FIG. 3. Lethal effect of ultraviolet radiation to isolates of *C. sativus*.

Many isolates appeared less pathogenic and a few were more pathogenic than their respective parents. Frequently the pathogenicity of morphological mutants was unchanged. Some of the weakly pathogenic mutants, however, did not sporulate and were sexually "infertile".

Occasionally perithecia were observed in some crosses between poorly sporulating, weakly pathogenic, white-spored strains and a densely sporulating, highly pathogenic, dark-spored strain. Tetrads (Fig. 5) isolated from black perithecia of such matings failed to demonstrate clearly segregating factors for pathogenicity (Table I). Although the parents differed markedly (Fig. 6), the progeny, which were divided into tetrads on the basis of the similar morphology of sister isolates, exhibited levels of virulence comparable with that of the dark-spored parent, A18. The amount of sporulation appeared to segregate 1:1. Pathogenicity of the isolates, therefore, was not dependent upon the amount of sporulation.

TABLE I

Comparative disease ratings of tetrads (average of eight replicates) and parental isolates (average of four replicates) of *C. sativus* to Thatcher wheat seedlings

| Conidial color | Sporulation | Tetrads | | | |
|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 |
| White | Sparse (parent) | 7 | 6 | 11 | 15 |
| Dark | Abundant (parent) | 36 | 41 | 41 | 56 |
| White | Abundant | 35 | 43 | 41 | 58 |
| White | Sparse | 30 | 40 | 42 | 48 |
| Dark | Abundant | 32 | 40 | 38 | 51 |
| Dark | Sparse | 32 | 31 | 42 | 50 |

Of the mutants screened for nutritional deficiencies one only developed more rapidly in MM supplemented with vitamins. In shake culture, yield increases of 25% were obtained with this mutant through vitamin fortification of the medium.

## Discussion

The difference in resistance of the white and dark spores to ultraviolet radiation may result from differences in size and pigmentation. The dark spores appeared somewhat larger than the white and probably possessed thicker cell walls. The pigment in the walls of spores of the wild type likely afforded some protection from the injurious effects of ultraviolet rays. In this connection, Dillon-Weston (3) found that a pigment in the epispore of a normal uredospore of *Puccinia graminis tritici* Eriks. and Henn. protected it from injurious radiation emanating from a mercury-vapor sun lamp. He also reported (4) that light-colored uredospores (white and orange) were more susceptible to ultraviolet rays than red or greyish-brown spores of the same physiological race. Fulton and Coblentz (5) observed that of 27 species of fungi, including an unidentified species of *Helminthosporium*, that were exposed to ultraviolet radiation, the more resistant fungi, as a group, were the ones having dark walls. The variation in resistance of the spores of an isolate from test to test may be caused by the differences in thickness of cell wall and the density of the pigment, as suggested by Hollaender *et al.* (8) for *Aspergillus terreus* Thom.

Greaney and Machacek (6) reported that ultraviolet radiation increased the frequency of mutation in some isolates of *H. sativum*. The present work confirms this finding. The erratic increase in the numbers of mutants and subsequent decline with treatment perhaps may be attributed in part to the multicellular and multinucleate nature of the conidia. Generally, spore germination is bipolar, occasionally unipolar. Upon the death of a terminal cell, however, germination may occur from the adjacent intercalary cell (unpublished data). The visible expression of a mutation within a terminal cell may be more rapid than from within an intercalary cell, presuming that germination occurred from the remaining terminal cell. Further, although multipolar germination is infrequent in *C. sativus*, observations indicate that it

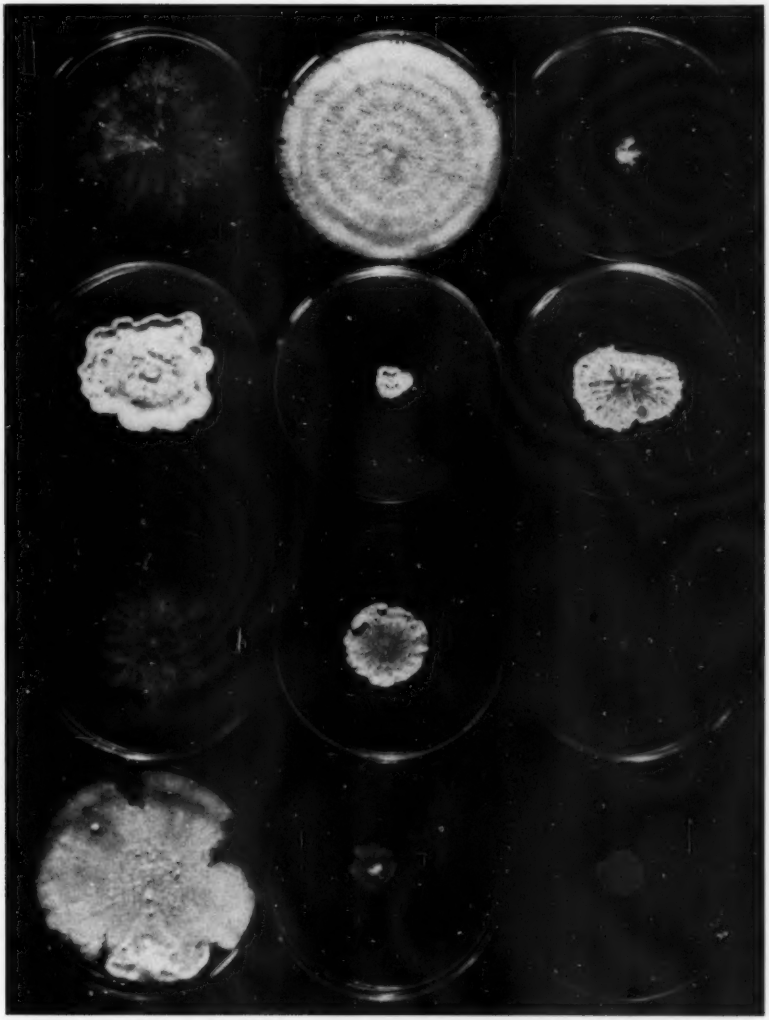

FIG. 4. Ultraviolet induced mutations in *C. sativus*. Parental isolate K3 on lower left.

PLATE III

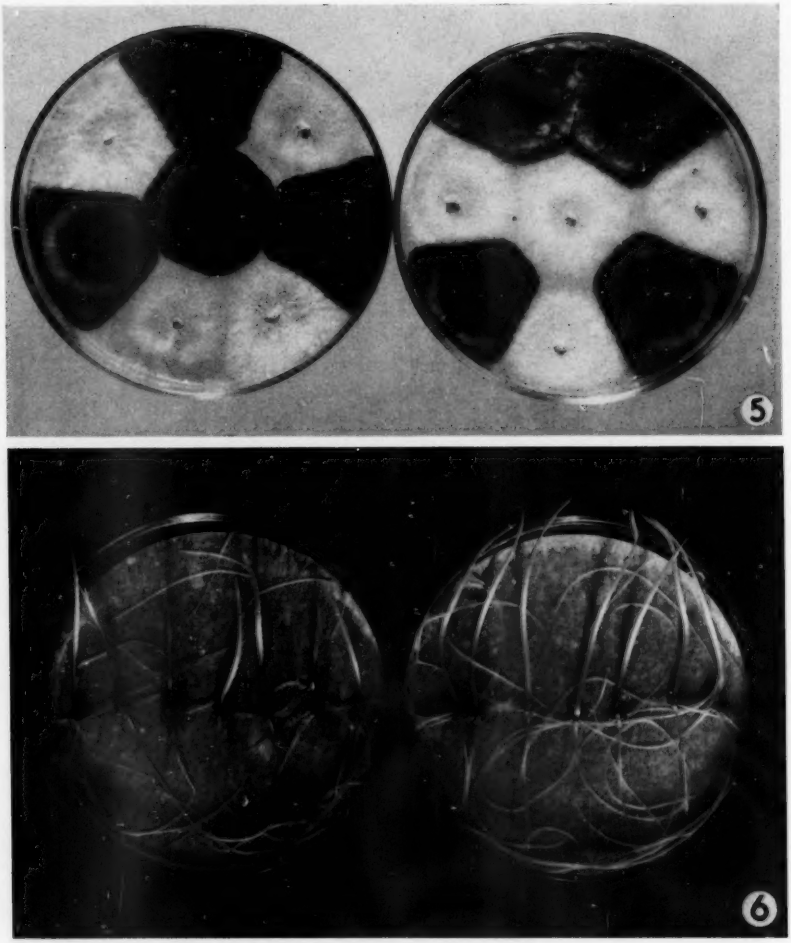


FIG. 5. Cultures of the eight ascospores from two asci of *C. sativus*. Note the four sister pairs of isolates forming a tetrad.

FIG. 6. Pathogenicity of compatible isolates of *C. sativus* to Thatcher wheat seedlings. Left, isolate A18, a monoascosporic, dark-spored strain; right, isolate No. 8, a mutant, white-spored strain.

occurs more commonly from white than from dark spores. This phenomenon may explain the high mutation rate recorded with the white-spored strain. The phenotypic expression of a permanent change in one nucleus may be masked by a normal development of the clone effected by numerous normal nuclei. Probably the apparent absence of mutants heterotrophic for a vitamin or amino acid may also be explained on this basis.

Some of the morphological mutant characters obtained during this study may be useful as markers in inheritance and ecological studies with the organism. Those apparently associated with altered virulence are of special interest. Unfortunately many of the mutants tested seemed sexually infertile. Such infertility may be due to chromosomal aberrations with resulting incompatibility of chromosomal pairing when the mutants were mated with strains of opposite reaction. Although tetrads exhibited a level of pathogenicity similar to that of the female parent, cytoplasmic inheritance apparently was not involved (unpublished data). Possibly multiple factors or hetero alleles control the character. Presumably through mutation, selection, and breeding, strains may be obtained which when tested on a number of hosts will clarify the parasitic specialization in *C. sativus* and, in addition, be useful for evaluating host resistance.

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# STUDIES ON RUBUS VIRUS DISEASES IN BRITISH COLUMBIA

## VI. VARIETAL SUSCEPTIBILITY TO APHID INFESTATION IN RELATION TO VIRUS ACQUISITION[1]

RICHARD STACE-SMITH


### Abstract

Field populations of the large raspberry aphid, *Amphorophora rubi* (Kalt.), were observed for 4 years on 13 varieties of raspberry. Lloyd George and St. Walfried were immune, Malling Enterprise and Malling Promise were resistant, and the remainder were susceptible to infestation. Each variety was naturally infected with black raspberry necrosis virus. Vector studies demonstrated that this virus was acquired with difficulty from the immune varieties and also from the susceptible variety St. Regis, but was readily acquired from the remaining 10 varieties.


### Introduction

The large raspberry aphid, *Amphorophora rubi* (Kalt.), is the most serious and widespread vector of virus diseases affecting raspberries. Large aphid populations are found on some varieties, sparse populations on others, and still other varieties are immune from aphid infestation. An experiment was conducted to determine whether aphids are able to acquire viruses more readily from some varieties than from others and, if so, whether there is any correlation between varietal susceptibility to infestation and virus acquisition by the aphid.

### Materials and Methods

The varieties tested were grown in a raspberry trial plot on the campus of the University of British Columbia. The plot contained 13 varieties, 6 of North American and 7 of European origin. Aphid populations were recorded by counting the number of aphids on partially expanded leaves below the tip of the new canes. Counts were made at intervals throughout the growing season in the 4 years 1954 to 1957.

All of the varieties in this plot were naturally infected with black raspberry necrosis virus. This virus is mild or symptomless in red raspberry varieties but induces severe symptoms in black raspberry (3). To test virus acquisition, 100 or more non-viruliferous aphids were transferred from a stock colony to a detached leaf of the test variety. The leaf was placed in a moist Petri dish and the aphids were given an acquisition feeding period of 16 hours. Fifty aphids from each source were transferred to healthy black raspberry seedlings, one aphid per plant, for a test feeding of 1 hour, and then destroyed. Only those aphids were used that were observed to be in the feeding position. The test was repeated for the three varieties showing low transmission (Table I).

[1]Manuscript received January 29, 1960.
Contribution No. 15 from the Research Station, Canada Department of Agriculture, Vancouver, B. C.

## Results

Aphid populations fluctuated considerably throughout the season and from year to year, but, in general, resistant varieties had consistently lower populations than susceptible varieties. Two varieties, Lloyd George and St. Walfried, were immune. No aphids were found on these varieties at any time during the four growing seasons. Two other varieties, Malling Enterprise and Malling Promise, had sparse populations. At the peak of aphid infestation, which was usually about the last week in June, an average of less than two aphids per leaf was found on these varieties, which were designated resistant. The remaining nine varieties showed a range of infestation and were all considered susceptible. An average of four or more aphids per leaf was found on these varieties. The highest populations were found on Cuthbert, Newburgh, and St. Regis, where the average was over 10 aphids per leaf at the peak of infestation. This varietal response to infestation is summarized in Table I.

In those varieties that were immune from the aphid, Lloyd George and St. Walfried, transmission was very low, but transmission was also low from one of the susceptible varieties, St. Regis. Transmission from the remaining 10 varieties was relatively efficient. These results are summarized in Table I.

TABLE I

Susceptibility to aphid infestation and virus transmission from 13 raspberry varieties

| Variety | Susceptibility | Transmission* |
|---|---|---|
| Lloyd George | Immune | 2/100 |
| St. Walfried | Immune | 1/100 |
| Malling Enterprise | Resistant | 13/50 |
| Malling Promise | Resistant | 24/50 |
| Cuthbert | Susceptible | 26/50 |
| Latham | Susceptible | 16/50 |
| Malling Landmark | Susceptible | 22/50 |
| Newburgh | Susceptible | 23/50 |
| Norfolk Giant | Susceptible | 18/50 |
| Seedling G | Susceptible | 24/50 |
| St. Regis | Susceptible | 2/100 |
| Taylor | Susceptible | 14/50 |
| Washington | Susceptible | 27/50 |

*Numerators indicate the number of transmissions; denominators indicate the number of plants inoculated.

## Discussion

The practical value of incorporating aphid resistance into a raspberry breeding program as a means of controlling virus diseases was demonstrated in 1938 by Huber and Schwartze (1). They found that *A. rubi* failed to reproduce on the variety Lloyd George and that resistance to this aphid was an inherited characteristic. The limited observations reported in this paper indicate that other sources of resistance are available. Certainly the variety St. Walfried, since it appears to be immune from the aphid, would be of value. Malling Enterprise and Malling Promise, which are resistant, may also be useful. The two immune and two resistant varieties are derived from the

European red raspberry, *Rubus idaeus* L., whereas the susceptible varieties are derived primarily from the American red raspberry, *R. idaeus* subsp. *strigosus*. It appears that resistance to the American strain of raspberry aphid is derived from the European red raspberry; recent work by Knight *et al.* (2) suggests that the converse is true in Europe.

It is not known why aphids can acquire the virus more readily from some varieties than from others. These results demonstrate that virus is acquired with difficulty from varieties that are naturally immune from the aphid. This may be explained by the fact that aphids feed fitfully when confined on apparently distasteful varieties and thereby fail to acquire an infective charge of the virus. The very low transmission from the susceptible variety St. Regis, however, cannot be explained on this basis; some unknown factor is responsible for the abnormal transmission from this variety.

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# FACTORS AFFECTING SUBSEQUENT GERMINATION OF CEREAL SEEDS SOWN IN SOILS OF SUBGERMINATION MOISTURE CONTENT[1]

H. A. H. WALLACE


## Abstract

Seeds of wheat sown in soils of subgermination moisture content sometimes decay and die. The critical moisture content at which maximum seed decay occurs is at a level approximately equal to one-half the difference between air-dry soil and soil moist enough for seed to germinate. The relationship apparently holds irrespective of soil type, even though the actual moisture percentage of different soil types at the critical level is quite distinct. "Dry" soil as used in these studies refers to air-dry soil with 8% moisture added. The subsequent germination of wheat was reduced after 3 days' incubation in "dry" soil at 30° C, and 14–20 days at 5° C. Germinability was reduced in strongly saline soil. Different samples of wheat varied greatly in germinability after incubation in "dry" soil. Sterilizing the "dry" soil did not change its effect on germination of various seed lots.

Much of the variation in loss of germinability was correlated with thresher injuries to the seed coat. Wheat, rye, and hull-less varieties of oats and barley, especially with seeds of more than standard weight per bushel, are susceptible to thresher injury. Growth cracks, sprouting, and frost injury all affect germination adversely. Cereal seeds with hulls, or with high moisture content, or below standard weight, or of small size, and wheat without projecting embryos, are less susceptible to thresher injury, and to consequent reduction of germinability.

The "field" fungi *Alternaria* sp. and *Helminthosporium* spp. grew out of cereal seeds plated on potato-sucrose agar or moistened filter paper and from germinated seeds grown in "moist" soil. After incubation in "dry" soil germinating seeds gave rise to *Alternaria*, but not to *Helminthosporium* spp. The non-germinating seeds from "dry" soil were infected by "storage" fungi, e.g. *Penicillium*, *Aspergillus*, *Rhizopus*, and *Mucor*. The "storage" fungi do not usually infect cereal seeds sown in "moist" soil, but in "dry" soil both sound and injured seed can be infected. The infection of sound seed is slow, permitting the seed to germinate, but injured seed is infected rapidly and does not germinate. The "storage" fungi invade seeds already infected by "field" fungi and inhibit the growth of the latter.

All samples of treated and untreated wheat seed sown in "moist" soil gave good germination. After incubation in "dry" soil the germination of treated seed was fair to good, and of untreated seed was poor to good. Treatment with formalin before incubation in "dry" soil doubled the germination; treatment with Ceresan M, Half-ounce Leytosan, and a water soak trebled germination compared with the untreated check. However, the best treatment in "dry" soil gave 31% less germination than the untreated check sown in "moist" soil. Under "dry" soil conditions a sound seed coat appears to provide better protection against seed-decaying organisms than any seed treatment tested.


## I. Soil Conditions

### INTRODUCTION

In an experiment to determine the minimum soil moisture required to germinate wheat it was found that seed sown in soils of subgermination moisture content often germinated poorly when ample moisture was supplied later. Since no published information was found on the subject a series of experiments

[1]Manuscript received December 7, 1959.
Contribution No. 35 from the Canada Department of Agriculture Research Station, Plant Pathology Laboratory, Winnipeg, Manitoba.

was carried out. Several methods of determining germination in soils of sub-germination moisture content were compared. The effects of various soil moistures, temperatures, length of incubation period in "dry" soil, the effect of the physical condition of the soil, of soil salinity, and of sterilizing the soil were determined, and are reported in Part I of this paper.

## Materials and Methods

### *Germination Tests in "Dry" Soil*

Air-dry potting soil composed of 4 parts loam and 1 part sand was weighed and moistened with a known amount of water, usually about 8% of the air-dry weight. The soil was then sifted through a 2-mm sieve and thoroughly mixed. This soil, too dry to permit seed germination, is referred to in this paper as "dry" soil. A typical air-dry sample tested by R. A. Hedlin, Associate Professor, Department of Soils, University of Manitoba, had a hygroscopic moisture of 4.23% and a moisture equivalent of 29.7%. Therefore, when 8 g of water were added to 100 g of air-dry soil, the moisture content was 43% of the moisture equivalent.

A modification of the Briggs and Shantz (2) method for determining the lower limit of available soil moisture was used in the first group of tests. Four-inch pots which had been dipped in paraffin were filled with "dry" soil and 30 to 50 seeds were sown in each pot. The soil surface was then sealed with melted paraffin, the pots were placed on a greenhouse bench, and covered with kraft paper. After 9 days at about 20° C (unless otherwise stated) the paraffin was removed from the surface, and the soil was sifted through a 2-mm sieve to recover the seeds. Adhering soil was removed from the seeds, which were placed on moist filter paper in Petri dishes to germinate. Seed were considered "germinated" when a normal shoot and one or more seminal roots were formed. Seeds were removed daily as they germinated for a period of 1 week. Excessive mold growth made it impossible to continue the tests longer than 1 week.

A simpler method was used in later experiments. A layer of "dry" soil was placed in Petri dishes and 50 seeds were sown on its surface. The seeds were covered with the same soil, and the Petri-dish cover was pressed down to pack the soil. It was not necessary to seal the edges of the dishes. After 9 days the seeds were recovered and plated on moist filter paper as with the previous method.

The germination results obtained by the waxed-pot and Petri-dish methods were checked in a large seed bed (11 ft by $2\frac{1}{2}$ ft) with a 4-in. depth of soil. Soil in this bed was thoroughly mixed and allowed to dry until its moisture content approximated that of the specially prepared "dry" soil. The seeds were sown in rows, covered, the soil firmly packed, and a layer of wax paper was put over the bed to reduce the loss of soil moisture. Nine days later the wax paper was removed and the seed bed watered. Emerged seedlings were counted after 8 days.

*Germination Test in "Moist" Soil*

The non-sterile soil method developed by Machacek and Wallace (15), here referred to as the "moist" soil germination test, was used as a control. By this method, the seed was sown on the surface of water-soaked soil, covered with a half-inch of sieved soil, and then lightly watered as needed. Counts of emerged seedlings were made 8 to 10 days later.

## Experimental Results

*Effect of Soil Moisture Content on Germination and Emergence of Wheat*

As the minimum soil moisture required for germination of wheat seeds was not known, six lots of soil were prepared containing respectively from 3 to 20% moisture (air-dry basis). Four waxed pots were filled with soil of each moisture content and seeded with wheat. The seed for one pot was treated with New Improved Ceresan, and the other three samples were untreated. The pots were sealed with paraffin and placed in the greenhouse; emergence counts were made 18 days later. As shown in Table I all seeds emerged from soil with 20% moisture, some from soil with 15% moisture, but none from the drier soils. The seedlings had no difficulty breaking through the paraffin seal. Ungerminated and unemerged plants were recovered by screening the soil. The ungerminated seeds were wiped clean and weighed. The weights indicated (Table I), and later experiments confirmed, that seeds absorb water in direct relationship to the moisture content of the soil.

TABLE I

Emergence and weight of ungerminated wheat seeds 18 days after seeding in soils of different moisture content (means of four replicates)

| Soil moisture, % (air-dry basis) | Emergence,* % | Weight of ungerminated seeds, g |
|---|---|---|
| 3 | 0 | 0.88 |
| 6 | 0 | 0.95 |
| 9 | 0 | 1.01 |
| 12 | 0 | 1.13 |
| 15 | 70 | — |
| 20 | 100 | — |

*Thirty seeds were sown in each pot.

The ungerminated seeds were placed on moist filter paper and germination counts were made a week later (Table II). The low germination of untreated seed (36.3%) from the soil having 9% moisture was not anticipated and led to the experiments described in this paper. The great increase in germination due to seed treatment led to other experiments described in Part IV.

An attempt was made to determine the effect of the period of incubation of wheat seeds in "dry" soil on their subsequent germination. "Dry" soil was placed in pots, sown with wheat, sealed with paraffin, and kept at 20° C. The seeds were recovered from four pots of soil at intervals of 2 days and tested for germination on moist filter paper. For "0" days, and as a check for each

TABLE II

Germination of wheat on moist filter paper after incubation in soils of low moisture content for 18 days

| Soil moisture, % | Germination, % | |
|---|---|---|
| | No treatment (mean of three replicates) | New Improved Ceresan (one replicate) |
| 3 | 90.1 | 100.0 |
| 6 | 88.1 | 100.0 |
| 9 | 36.3 | 85.8 |
| 12 | 84.8 | 95.7 |
| 15 | 100.0* | 95.7 |
| Control† | 90.1 | — |

*Includes seedlings that had emerged in the soil test.
†Seeds sown directly on filter paper.

TABLE III

Effect of number of days of incubation in "dry" soil on subsequent germination of wheat (means of four replicates)

| Number of days in "dry" soil | Germination, % | |
|---|---|---|
| | "Dry" soil test | Control test on filter paper* |
| 0 | 79.6 | 90.8 |
| 2 | 79.6 | 88.4 |
| 4 | 73.6 | 94.1 |
| 6 | 56.1 | 87.5 |
| 8 | 39.6 | 84.2 |
| 17 | 30.7 | 84.2 |
| L.S.D. 5% level | 10.3 | 10.3 |

*Seeds contaminated with "dry" soil and then placed on filter paper.

time interval, the seed was merely contaminated with soil. A significant reduction in germination occurred after 6 days' incubation in "dry" soil (Table III). Prolonging the incubation period caused a further reduction in germination.

The results of other experiments indicated that the minimum incubation period required for a reduction in germination varied with the temperature, being 3 days at 30° C, and 14 to 20 days at 5° C.

As a result of these and other tests a standard "dry soil germination test" using 8% soil moisture (air-dry basis) and an incubation period of 9 days at room temperature (about 20° C) was established.

*Physical Condition of the Soil*

A range of moisture content was set up in each of four soils (fine sand, fine sandy loam, a greenhouse mixture of 4 parts loam and 1 part sand, and clay loam). Wheat was sown in each soil and incubated for 9 days. Later the seeds were recovered and tested for viability. The lowest percentage germinations occurred after incubation at 1.5, 6.0, 8.0, and 11.0% moisture in the four soils, respectively. These results indicate that maximum loss of viability of wheat seeds in any one soil occurs at about one-half the minimum soil moisture required for seed germination in that particular soil.

*Salinity of the Soil*

Ayers (1) found that germination of vegetable seeds was reduced in saline soils when the moisture content approached the wilting point. Machacek (11), however, obtained no appreciable reduction in germination of wheat due to magnesium sulphate in solution at concentrations up to 2.57%.

To test the effect of soil salinity on germination of wheat incubated in "dry" soil, experiments were made with various concentrations of calcium sulphate and of magnesium sulphate, respectively. The salts were added to air-dry soil in quantities equivalent to 0.00 (check), 0.75, 1.50, and 2.25% by weight of soil, in distilled water to bring soil moisture to 8%.

Subsequent germination of wheat seeds incubated in these soils indicated that 2.25% calcium sulphate caused a significant reduction in germination (Table IV). Although magnesium sulphate showed a trend similar to that obtained for calcium sulphate, the results were not statistically significant.

*Sterile and Non-sterile Soil*

Samples from nine different lots of disease-free wheat were sown in soil that had been autoclaved and then dried in a warm oven, and in similar unsterilized air-dry soil. Both lots of soil were adjusted to the "dry" soil

TABLE IV

Germination of wheat after incubation in "dry" soil containing various concentrations of calcium and magnesium sulphates (means of seven replicates)

| Concentration of salt, % | Germination, % | |
|---|---|---|
| | Soil with $CaSO_4.2H_2O$ | Soil with $MgSO_4.7H_2O$ |
| 0.00 | 50.9 | 47.1 |
| 0.75 | 54.0 | 60.3 |
| 1.50 | 44.9 | 53.4 |
| 2.25 | 36.3 | 48.6 |
| L.S.D. ($P = 0.05$) | 8.0 | Not significant |

TABLE V

Germination of different samples of Redman wheat in "moist" soil and germination after incubation in unsterilized and sterilized "dry" soil

| Sample No. | Germination, % | | |
|---|---|---|---|
| | "Moist" unsterilized soil | "Dry" soil | |
| | | Unsterilized | Sterilized |
| 1 | 87 | 52 | 40 |
| 2 | 89 | 46 | 46 |
| 3 | 93 | 70 | 68 |
| 4 | 95 | 100 | 100 |
| 5 | 96 | 46 | 46 |
| 6 | 97 | 68 | 74 |
| 7 | 99 | 62 | 84 |
| 8 | 100 | 94 | 98 |
| 9 | 100 | 96 | 98 |

moisture content by the addition of sterile water. Seed was also sown in unsterilized "moist" soil as a control. Germination was 87 to 100% in "moist" soil (Table V) and 40 to 100% after incubation in "dry" soil. Germination after incubation in sterilized and unsterilized "dry" soil was about the same.

As shown in Table V, there were striking differences in the results with various seed samples. These results indicate that the factors responsible for the death of the seed are not necessarily present in the soil; they may be in or on the seed.

### Discussion

Cereal seeds are normally sown in moist soil. However, the farmer is often compelled to sow cereals in dry soil. For example, the growth of cereals in northern latitudes is limited by the number of frost-free days, so the tendency is to sow early, even if the soil is dry. In heavy clay soils, as in the Red River Valley of Manitoba, the threat of late spring rains also puts pressure on the farmer to sow early. The common practice of allowing wild oats to germinate and then sowing cereals late also may necessitate sowing cereals in a dry seed bed. Where winter cereals are grown, other factors often result in seed being sown in dry soil.

Some of the reasons for seeds failing to germinate after incubation in "dry" soil are indicated below.

"Straight", "tough", and "damp" classes of common wheat contain 14.5, 14.6–17.0, and over 17.0% moisture, respectively. The moisture contents of these classes are slightly different for other cereals. "Straight" grade wheat stores well. "Tough" grades slowly deteriorate in quality and viability. "Damp" grades become moldy and lose their viability. A minimum of 30.0% moisture, air-dry weight (optimum 36.0–40.0%), is required for germination of wheat.

Wheat incubated in soil having 9.0% moisture was 0.13 g heavier than similar seed incubated in soil of 3.0% moisture (Table I), an increase of about 15%. "Straight" grade wheat usually has 10.0–14.5% moisture. Therefore, the seed sown in soil of 9% moisture reached a moisture content of 25.0–29.5% which is not enough for the wheat to germinate, but enough to classify it as "damp" wheat. Such wheat usually loses its viability rapidly, as it did in the original experiment, but when other samples of wheat were tested the germination was variable (Table V). Since repeated tests with aliquots from individual samples gave relatively constant germination, even in unsterilized and sterilized soil, the variation between samples must be due to the condition of the seed.

## II. Seed Conditions

### Introduction

As shown in Part I variation in germination between samples must be due to the condition of the seed. This part presents some of the results of investigations on factors, associated with the seed, which affect germination.

## Experimental Results

*Kind of Crop*

Stocks of Elite and Foundation wheat, oats, barley, and rye seed of the 1949 and 1950 crops were obtained from all cereal-growing regions of Canada and subjected to the "dry" soil germination test. Preliminary tests had indicated that seed of these crops responded in the same way to changes in temperature and moisture conditions of the soil. As shown in Table VI, the average germination percentages for wheat and rye in "dry" soil were considerably lower than those for oats and barley. Germination tests of commercial grain gave similar results. Apparently "seed" grain germinates no better than ordinary commercial grain under "dry" soil conditions.

Preliminary tests with corn, peas, and beans indicate that large-seeded crops germinate better than do cereal crops after exposure to dry soil conditions, but the germination of small-seeded crops, such as flax and alfalfa, is usually affected even more than the cereals.

TABLE VI

Germination of Elite and Foundation seed of cereal crops by the "moist" and "dry" soil tests

| Crop | Year of production | No. of samples | Mean germination, %: "Moist" soil | Mean germination, %: After incubation in "dry" soil |
|---|---|---|---|---|
| Wheat | 1949 | 138 | 92 | 65 |
| | 1950 | 138 | 89 | 55 |
| Oats | 1949 | 68 | 92 | 79 |
| | 1950 | 67 | 89 | 80 |
| Barley | 1949 | 54 | 91 | 79 |
| | 1950 | 51 | 94 | 80 |
| Rye | 1949 | 2 | 76 | 60 |
| | 1950 | 2 | 84 | 20 |

*Variability between Samples*

Examination of detailed data for germination of the wheat samples brings out (Table VII) the following facts: (*a*) a few samples in each year gave less than 20% germination by the "dry" soil test, although germination in "moist" soil was fair to very good; (*b*) more than half of all the samples tested germinated 60% or less after incubation in "dry" soil; (*c*) the mean germinability of the seed after incubation in "dry" soil differs from year to year.

*Hulled vs. Hull-less Seed*

The germination of wheat and rye in "dry" soil tests was considerably lower than that for oats and barley (Table VI). When five hull-less and five hulled varieties of oats grown together at the same station were subjected to the "dry" soil test the range of germination in the former was 10 to 44% and in the latter 70 to 92%. Smith (20) found that removal of the hull of

TABLE VII

Differences in germination of samples of Elite and Foundation wheat seed of the 1949 and 1950 crops in "dry" and "moist" soil tests

| Germination (%) in the "dry" soil test* | No. of samples in each category | | Mean germination (%) of same samples in the moist soil test | |
|---|---|---|---|---|
| | 1949 | 1950 | 1949 | 1950 |
| 1– 20 | 6 | 3 | 90 | 76 |
| 21– 40 | 27 | 32 | 90 | 84 |
| 41– 60 | 22 | 55 | 91 | 91 |
| 61– 80 | 31 | 29 | 90 | 90 |
| 81–100 | 51 | 18 | 95 | 98 |
| Mean germination | | | 92 | 89 |

*Mean germination in the "dry" soil test was 65% for the 1949 seed and 55% for the 1950 seed.

cereal seeds sometimes improved germination, but the number of moldy kernels was greatly increased. Possibly hull-less varieties, like Smith's dehulled seeds, are more subject to attack by fungus.

*Source of Seed*

The germination after incubation in "dry" soil of 25 samples of wheat from each crop inspection district in Canada ranged from 33 to 64%. The cause of this variability was not determined. Perhaps weather conditions at harvest affect the germinability of the seed in "dry" soil, possibly by their effect on the amount of mechanical injury to the seed.

*Damage to the Seed Coat*

It has been indicated above that a great variation in germinability in the "dry" soil test exists between samples of seed, and that the hull provides some protection to the seed. Visual examination of a few wheat samples that had germinated poorly in the "dry" soil test revealed that many seeds had the seed coat ruptured over the embryo. Russell and Ledingham (19) and Mead *et al.* (17) found that germination was lowered when a large proportion of seeds had their embryos exposed, particularly when the soil moisture was limited. Mead *et al.* describe two types of embryo exposure: (*a*) the pericarp and adjacent layers are broken off exposing the tip of the coleorhiza; (*b*) fractures in the pericarp and the seed coats over the face of the embryo. Noll (18) using hand-picked seeds from heads of wheat was unable to infect them with *Penicillium* unless he injured them on the dorsal side. Tirelli and Scaramuzzi (21) found the greatest reduction in germination when the seed-coat injuries were near the embryo, provided these injuries were deep enough to reach the tegument. Hurd (8) observed that an unbroken seed coat ordinarily affords absolute protection against attack of living seeds by *Penicillium* or *Rhizopus* in damp storage, in the soil, and on moist blotting papers, except at low temperatures or when the vitality of the seed was impaired by seed treatment. When the break occurred over the endosperm all infections were fatal to the seed, but when it was over the embryo, the seed remained practically unharmed.

All workers cited agree that injury of the seed coat affects germination adversely, although there is some difference of opinion regarding the effect of location of the injury. However, their observations were limited to seed germination under "moist" conditions and did not extend to the "dry" soil conditions used by the writer.

To determine the effect of location and type of injury to the seed coat of wheat on its germination after incubation in "dry" soil, wheat seeds examined with a low power microscope were separated into three lots, the first showing embryo exposure, the second showing growth cracks in the cheeks over the endosperm as described by Ledingham (10), and the third showing no injury. The last lot was divided into four parts of which one was kept undamaged, and the other three damaged as follows: (*a*) the pericarp over the embryo was torn open with a dissecting needle, (*b*) the tip of the embryo projection was cut off with a knife, and (*c*) a cut was made in the dorsal side of the seed so as to expose the endosperm.

The sound and the damaged seed germinated equally well in "moist" soil, but 10% of the seeds with their embryo projections cut off were infected by *Penicillium* spp. and produced stunted seedlings. In the "dry" soil test the sound seed germinated 60%, seed with dorsal exposure of the endosperm germinated 16%, and seeds with other types of injury germinated 4% or less. These results indicate that injury to any part of the seed can affect its germination when it is sown in "dry" soil.

The samples of Elite and Foundation wheat seed previously mentioned were examined microscopically for mechanical injury at the embryo region of the seeds. In the 1950 crop the coefficient of correlation between percentage of injured seeds and percentage germination in "moist" soil was $-0.328$ and in "dry" soil it was $-0.505$. Both coefficients were statistically significant, and were similar to the coefficients obtained for the same data for the 1949 crop. However, the mean reduction in germination due to seed injury in "moist" soil was only 2 and 3% compared with 30 and 35% reductions in "dry" soil for the 1949 and 1950 crops, respectively. Higher correlations were not obtained because a small percentage of injured kernels in the sample affected germination more than seemed warranted by the amount of injury. Although the reason for this effect is not known, it may be due to minute undetected injuries to the seed coat. This hypothesis is supported by the fact that samples of seed containing no injured kernels germinated very well, but samples containing 1 to 10% injured seeds frequently showed 20 to 30% reduction in germination after incubation in "dry" soil.

*Variety of Wheat*

Russell and Ledingham (19) found the mean embryo exposure for samples of Thatcher, Marquis, and Renown wheat to be 19.4, 22.1, and 35.5%, respectively. The writer obtained six varieties of wheat from 20 stations in Western Canada and examined them for mechanical injury. The mean injury to Thatcher, Redman, and Selkirk was 27, 51, and 54%, respectively. It has been suggested by Mead *et al.* (17) that these varietal differences are

due to variation in the length of the embryo projection. The Elite and Foundation seed obtained in 1949 had 15 and 27% mechanical injury in Thatcher and Redman wheat, respectively, and their respective germinations after incubation in "dry" soil were 80 and 42%.

*"Frosted" and "Sprouted" Seed*

Frost either kills the developing seed (frozen grain) or merely injures the seed (frosted grain). "Frosted" grain usually germinates, but seedling growth is retarded and there is a waviness of the veins and a twisting of the primary leaf (15). As "frosted" seeds generally have wrinkled seed coats, they might be particularly prone to thresher injury and consequent reduction in germinability. Four hundred and twenty-five samples of wheat were tested for the presence of "frosted" seed by growing them in moist soil. Samples containing "frosted" kernels proved to have 8.5% more embryo exposure than samples with no "frosted" seed.

As the seed coat over the embryo of "sprouted" seeds is ruptured, "sprouting" is a type of embryo exposure. Twenty-five samples of winter wheat, representing a wide range in the percentage of sprouted kernels, germinated 91 and 39% when tested by the "moist" and "dry" soil methods respectively. The coefficient of correlation between the percentage of "sprouted" seeds and percentage germination in the "dry" soil test was $-0.650$. When percentage germination was correlated with the percentage of "sprouted" seeds plus the percentage showing embryo exposure due to thresher injury, the coefficient was $-0.813$. Both coefficients were highly significant.

*Weight per Bushel*

The writer has shown (22) that wheat weighing 57 lb per bushel had 20% less embryo exposure and 20% higher germination in the "dry" soil test than seed weighing 64 lb per bushel. Other data indicated that lighter seed, if not damaged by frost, would germinate even better. Since non-frosted light seed was not available, wheat containing "frosted" and "frozen" kernels had to be used. To eliminate the effect of non-viable seeds the germination in "dry" soil tests was expressed as a percentage of the germination in "moist" soil. The mean germination in "dry" soil tests expressed in this manner for the three groups of 25 samples weighing 48, 57, and 64 lb per bushel was 84, 72, and 46%, respectively.

*Statutory and Commercial Grade*

Machacek and Wallace (16), studying germination in cereal grain of different market grades, found that some samples of grain of the lowest grade germinated as well as the best samples in the highest grade. Where low germination occurred, the seed had been injured by frost.

Samples of 425 carloads of wheat were obtained from the Grain Inspection Office "Western Division", Board of Grain Commissioners, Winnipeg, Canada. These samples had all dried to a uniform moisture content before being tested for germination by the "moist" and "dry" soil methods. The percentages of

kernels showing embryo exposure were 41, 26, and 14 for "straight", "tough", and "damp" wheat, respectively. Within each of these categories grade No. 5 showed the least embryo exposure, but, on account of frost injury in grain of low grade, the highest germination in "dry" soil was found in tough or damp No. 2 and No. 3 Northern wheat. The mean germination of "damp" No. 6 wheat in "dry" soil, expressed as a percentage of the mean germination in "moist" soil, was 75%. For "straight" No. 1 Northern it was only 48%. Apparently, if frost had not been a factor, "damp" No. 6 wheat would have germinated the best after incubation in "dry" soil.

## Discussion

Good seed must be true to variety and free from disease, must germinate well, and must grow vigorously. Field inspections and laboratory tests ensure the production of "seed" grades. However, "seed" wheat sown in soils of subgermination moisture content may germinate poorly after ample moisture is made available. Although all cereals are affected, the reduction in germination is more pronounced in wheat and rye than in oats and barley. The loss in germination is due to seed-coat injuries caused by threshing and subsequent handling, or is due to natural causes such as growth cracks, frost, and sprouting. Oats and barley are less susceptible to injury because of the protection of the hull. Even so, the malting industry considers it necessary to publicize precise instructions for threshing barley because peeled and broken kernels affect the germination. The experiments reported here indicate that more attention should be given to the threshing of all "seed" crops, and especially to wheat and rye.

Since injury to any part of the seed affects its germinability by the "dry" soil test, the shape and size of the seed is particularly important. The long thin kernels of rye and the large seeds of durum wheat are particularly susceptible to injury. Some varieties of wheat are more liable to mechanical injury than others. For example, the long embryo projection of kernels of Apex wheat is easily broken. Apex, and to a lesser extent Selkirk wheat, are susceptible to mechanical injury. It would be difficult to grow sound seed of these varieties in dry areas. Similarly, the planting of these varieties in dry areas, because of the likelihood of injured seed germinating poorly, is undesirable if alternate varieties are available. The importance of kernel shape should be considered by plant breeders.

Large seeds are supposed to germinate better and produce more vigorous seedlings, but this is true only under ideal moisture conditions. When sown in a "dry" seed bed the germination may be low and some of the seedlings stunted. There is a need for more emphasis on soundness of the seed and less on its size. As shown in Table VII a few samples of wheat germinated less than 20% by the "dry" soil tests. The farmer who plants this seed is taking an unnecessary risk when the soil is dry. In contrast sound seed deserves more recognition since it gives more reliable germination, even when small in size. Since the amount of injury in a sample of grain can be determined with a low power

microscope in a few minutes it is suggested that "seed grades" be examined as a routine procedure by testing laboratories. Such a practice would induce the farmer to produce sound seed.

Thresher injury can be controlled. Machacek and Brown (12) using a hand clothes wringer with rubber rollers showed, for the first time, that thresher injury of flax could be prevented. Later, Cunningham and Hannah (4) developed a roller thresher for experimental plots. The farmer can reduce thresher injury by threshing when the grain is slightly tough and by proper adjustment of the machine. By such methods, he can produce seed which will be more likely to germinate well even if sown in dry soil.

## III. Seed- and Soil-borne Fungi

### Introduction

Since the ungerminated seeds are moldy when recovered from sterile and unsterile soil of subgermination moisture content the infection must come from the seed.

The effect of embryo exposure on the fungus flora of the seed was determined by plating the seeds on potato-sucrose agar or on moistened filter paper; by growing seedlings from seed planted in "moist" soil, or from seed previously incubated in "dry" soil; and by plating non-germinating seed from "dry" soil. This part presents the results, and discusses the interaction of saprophytic and facultatively parasitic "field" fungi, and their interaction with "storage" fungi.

### Results and Discussion

*Types and Prevalence of Fungi in Seeds*

Fungi can infect seed of cereals in the field after seeding, or they can infect the new crop of seed when it is developing in the head, when it is in the stook or swath, or when it is stored under damp conditions in the granary.

Fungi which infect seeds as they develop in the head, except in the case of loose smuts, are usually isolated by plating surface-sterilized seeds on potato-sucrose agar. Both saprophytes and facultative parasites are found. Machacek *et al.* (13) plated 6024 samples of seed and found *Alternaria* in 55, 56, and 67% of the seeds of wheat, oats, and barley, respectively. They found *Cladosporium* in less than 3.0%, and *Aspergillus*, *Penicillium*, *Rhizopus*, and *Trichothecium* (= *Cephalothecium*) in less than 0.5% of the seeds.

When cereal seeds which were not surface-sterilized were incubated on moist filter paper in Petri dishes all the above genera of fungi and many others grew from the seeds. Tests made in this way with commercial samples of the 1950 crop of wheat yielded results similar to those obtained by Machacek *et al.* (13), except that *Cladosporium* and *Trichothecium* occurred more frequently. These fungi are probably superficial and therefore killed by the usual surface sterilization of the seed.

When cereal seeds were plated after incubation in "dry" soil, *Alternaria* and *Cladosporium* were rarely present on non-germinating seeds. *Aspergillus*,

*Penicillium*, *Rhizopus*, and *Trichothecium* were found more frequently than on seed which had not been incubated in "dry" soil. These fungi are among those found on stored and "heated" grain. Christensen (3) found that most of the living mycelium beneath the pericarp of low grade lots of wheat was *Aspergillus* and *Penicillium*. In high grade lots most of the mycelium was *Alternaria*.

Hurd (8) found that injury to the seed coat over the endosperm (which is relatively uncommon) was always fatal if the seed was infected subsequently by *Penicillium* and *Rhizopus*, but if the seed was injured over the embryo (which is very common in wheat and rye) the seeds remained practically uninfected. The writer found that sound and injured seed germinated equally well in "moist" soil, but that any injury to any part of the seed coat was nearly always fatal after incubation in "dry" soil.

TABLE VIII

Comparative microflora of four samples of wheat before and after incubation in "dry" soil, as indicated by plating surface-sterilized seed on potato-sucrose agar

| Sample | Incubation | Embryo exposure, % | Germination, % | *Alternaria*, % | *Penicillium*, % | *Rhizopus*, % | Misc. fungi, % |
|---|---|---|---|---|---|---|---|
| A | None | 0 | 100 | 100 | 4 | 0 | 15 |
| | Dry soil | | 100 | 100 | 23 | 2 | 11 |
| B | None | 10 | 100 | 100 | 3 | 0 | 11 |
| | Dry soil | | 93 | 93 | 86 | 16 | 6 |
| C | None | 32 | 84 | 60 | 1 | 0 | 4 |
| | Dry soil | | 14 | 0 | 100 | 26 | 1 |
| D | None | 62 | 88 | 21 | 1 | 0 | 3 |
| | Dry soil | | 22 | 0 | 98 | 23 | 3 |

To determine what was killing the seed, four samples of wheat were incubated in "dry" soil in the usual way. After removal from the soil the seeds, and unincubated seeds from the same samples, were surface-sterilized and plated on potato-sucrose agar. The results (Table VIII) show that *Alternaria* was the most common fungus on unincubated seed, but that it decreased as the amount of embryo exposure increased. Other fungi were scarce. Seeds from "dry" soil gave high counts of *Alternaria* only in samples with good germination. Many of the ungerminated seeds were infested with *Penicillium* and some with *Rhizopus*. In some samples (e.g. sample B) seed showing high percentage of infection by *Penicillium* germinated well after incubation in dry soil. The percentage of seeds with exposed embryos was small in such samples. Apparently a sound seed coat, although it does not always prevent infection by *Penicillium*, hinders or retards it sufficiently that the embryo can survive and the seed can germinate. It is possible that *Penicillium* infection of apparently sound seed occurred through minute undetected injuries to the seed coat.

*Source of Infection*

Germination was similar for seed incubated in sterile and non-sterile "dry" soil. Therefore, if the fungi infecting ungerminated seeds were responsible for the death of the seeds, those fungi must have been carried either in or on

the seed. Since very low counts of *Penicillium* are usually obtained when seeds which have not been incubated in "dry" soil are surface-sterilized and plated on potato-sucrose agar, and high counts are obtained when non-surface-sterilized seeds are plated on similar agar, the fungus must be superficial. However, when unsterilized seeds are placed on moist filter paper in Petri dishes the occurrence of *Penicillium* is uncommon, except on heated or otherwise damaged seeds. It is presumed that the essential nutrients for rapid growth of *Penicillium* are supplied by the agar and that the testa or hull of cereal seed is a poor medium for the growth of the fungus. When the testa is broken a good medium for the growth of *Penicillium* is available. Infection is then governed by the available moisture. Abundant moisture favors development of the embryo, but limited moisture favors growth of the fungus. *Aspergillus*, *Rhizopus*, and *Trichothecium* behave in the same way as *Penicillium*. *Aspergillus* and *Mucor* occurred most abundantly on seeds incubated at temperatures over 80° F.

*Antibiotic Action of Seed-borne Fungi*

Machacek and Greaney (14) observed that *Helminthosporium sativum* inhibited development of *Alternaria tenuis* when both fungi are sown on agar. They suggested that when the developing seed is invaded by both fungi simultaneously, growth of *Alternaria* is hindered by *Helminthosporium*; that a seed already infected by *Alternaria* may be invaded, at least partially, by *H. sativum*; and that when both fungi are present on or in the seed the outward growth on agar of *Alternaria* is inhibited or retarded by the antagonism of *H. sativum*.

Since nearly all the unincubated seed was infected with *Alternaria* (Table VIII), the seed incubated in "dry" soil must also have been infected. The outward growth of this fungus must have been inhibited by *Penicillium*, as in samples C and D.

Since *Alternaria* infection of seed is usually considered as non-pathogenic, experiments were made with seeds infected with pathogenic fungi. Wheat and barley naturally infected with *H. sativum* and oats naturally infected with *H. avenae* were placed on moist filter paper and in "dry" soil for germination tests. On filter paper the germination was 68, 84, and 80% respectively, the reduced germination being caused by *Helminthosporium*. The germination after incubation in "dry" soil was 28, 42, and 56%, respectively, but *H. sativum* and *H. avenae* were not found. It appears that *Penicillium* and *Trichothecium* prevented the outward growth of both species of *Helminthosporium*, and that they and not *Helminthosporium* were responsible for the low germination of the seed.

## IV. Seed Treatments

### Introduction

Cereal seeds are commonly treated with chemicals to control the smuts and seed-borne pathogens causing seedling blights, such as *Helminthosporium*

spp. and *Fusarium* spp. The effectiveness of these treatments against the "storage" molds which can infect seeds sown in "dry" soil is unknown. However, when Machacek and Brown treated flax with New Improved Ceresan and Half-ounce Leytosan the percentage germination was consistently higher than for the untreated seed (12). The fungi infecting injured flax seed in "moist" soil and the fungi infecting cereal seed in "dry" soil appear to be the same. Chemical treatments ought to be effective for protecting cereals in "dry" soil, if the chemicals themselves are not injurious.

Julius Kühn (9), according to Falke (5), published a paper in 1872 on the injurious effects of copper vitriol on germination of thresher-injured seed. Falke himself tested copper vitriol and formalit (a formalin – copper chloride solution) and found that some varieties, especially of winter wheat from the 1903 crop, germinated very poorly after treatment. He attributed the low germination of the treated seed to poor development of the seed coat (especially over the embryo). The poor development he believed to be correlated with the very dry season of 1903.

Hurd (8) found that copper sulphate injured the germ of wheat in 3 to 5 minutes when the embryo was exposed, but that treatment for 1 hour was required to cause injury when the break in the seed coat was over the endosperm. The injury was characterized by a stunting of the roots.

Russell and Ledingham (19) found that embryo exposure permitted free access of formalin to the embryo and that this reduced seedling emergence and the yield of grain. They recommended the use of mercurial dust treatments for samples of seed containing a high percentage of embryo exposure.

Machacek and Wallace (16) observed hypertrophy of the coleoptile resulting from mercury poisoning of seedlings of wheat that had been treated with Ceresan M and stored for 30 days at room temperature. The correlation coefficient for the amount of the injury in wheat with the weight per bushel was +0.648. The writer, using the same samples and many others, found the coefficient of correlation between percentage embryo exposure and weight per bushel for wheat (of grades 1 Northern to No. 5) to be +0.656. Embryo exposure apparently facilitates mercury poisoning when the treated seed is stored for 30 days or more.

This part reports the effect on germination of wheat, oats, and barley when the seed is treated with New Improved Ceresan and sown in "moist" and "dry" soil. The effects of some standard seed treatments applied to mechanically injured wheat seed on the percentage germination after seeding in "moist" and "dry" soil are also presented.

## Experimental Results

*Experiment 1*

The non-sterile soil method for testing seed germination was used for the "moist" soil test (15). Samples of Foundation and Elite seed of the 1949 crop of Thatcher wheat, Victory oats, and Montcalm barley were used. It was not

TABLE IX

Effect of treatment with New Improved Ceresan on emergence of wheat, oats, and barley in "moist" and "dry" soil tests

| Soil condition | Crop | No. of samples | Mean emergence, % | | Difference in favor of treatment |
|---|---|---|---|---|---|
| | | | Treated | Untreated | |
| "Moist" | Wheat | 71 | 95.1 | 93.5 | +1.6 |
| | Oats | 16 | 83.4 | 83.8 | −0.4 |
| | Barley | 28 | 93.8 | 94.5 | −0.7 |
| "Dry" | Wheat | 71 | 86.5 | 74.0 | +12.5 |
| | Oats | 16 | 76.4 | 71.0 | +5.4 |
| | Barley | 28 | 90.8 | 81.8 | +9.0 |

TABLE X

Effect of New Improved Ceresan on emergence of Thatcher wheat after incubation in "dry" soil

| No. of samples in each range | Emergence, % | | | |
|---|---|---|---|---|
| | Untreated | | Treated, mean | Increase in emergence due to treatment, % |
| | Range | Mean | | |
| 1 | 21– 30 | 29 | 58 | 100 |
| 2 | 31– 40 | 35 | 67 | 91 |
| 3 | 41– 50 | 47 | 81 | 72 |
| 7 | 51– 60 | 56 | 74 | 32 |
| 14 | 61– 70 | 65 | 86 | 32 |
| 14 | 71– 80 | 75 | 85 | 13 |
| 10 | 81– 90 | 86 | 92 | 7 |
| 18 | 91–100 | 97 | 98 | 1 |

known at this time that Thatcher wheat was less susceptible to mechanical injury than other popular varieties. Each seed sample was divided into two subsamples, one of which was treated with New Improved Ceresan and talc (1 and 2 parts by volume, respectively) and the excess dust removed by screening, and the other part used without treatment. The germination counts of all crops made 10 days later (Table IX) showed very little difference between treated and untreated seed.

For the "dry" soil test the same large beds and the same treated and untreated seed were used as for the "moist" soil test. Mean germination after incubation in "dry" soil of treated wheat, oats, and barley was 12.5, 5.4, and 9.0% greater respectively than for untreated seed. The germination of various samples of wheat after incubation in "dry" soil is given in Table X in order to show the great variation between samples in response to seed treatment.

*Experiment 2*

The "moist" soil test was the same as above, but the "dry" soil test was modified as follows: the air-dried soil was moistened with the appropriate amount of water, mixed, and placed in Petri dishes. In this soil was sown a

sample of durum wheat, known to have exposed embryos, after it had received one of the following treatments: (*a*) no treatment; (*b*) soaking seed for 4 hours and 10 minutes in cold water, as a check on the hot water treatment; (*c*) soaking for 4 hours in water at 86° F, and then immersing for 10 minutes at 122° F to 126° F, as in the standard treatment for loose smut (7); (*d*) soaking in a 1/320 solution of formalin (40% formaldehyde) for 3 minutes, covering for 4 hours, then drying (6); (*e*) treatment with Ceresan M diluted with 2 parts talc; and (*f*) treatment with Half-ounce Leytosan, similarly diluted (15). Nine days after seeding in the Petri dishes the seeds were removed and planted in "moist" soil beds in the greenhouse. Germination counts were made 8 days later.

The germination of seed in all treatments, except hot water, was about the same in "moist" soil (Table XI). In contrast the germination of both treated and untreated seed by the "dry" soil test was much lower than for seed

TABLE XI

Effect of some standard seed treatments on germination percentage of wheat seed in "dry" and "moist" soil tests

| Treatment | "Dry" soil | "Moist" soil |
|---|---|---|
| No treatment | 19.5 | 95.0 |
| Hot water | 26.0 | 56.5 |
| Formalin | 37.0 | 90.5 |
| Cold water | 54.0 | 94.5 |
| Half-ounce Leytosan | 55.0 | 94.0 |
| Ceresan M | 64.0 | 94.5 |
| L.S.D. ($P = 0.05$) | 14.5 | 14.5 |

sown directly in "moist" soil. Furthermore, there was considerable variation between treatments. All treatments of the seed in the "dry" soil test increased the percentage germination, although the increase from the hot water treatment was not significant. Formalin treatment doubled, and the cold water soak, Ceresan M, and Half-ounce Leytosan trebled, the germination of the untreated seed sown in "dry" soil.

## Discussion

The experiments described in this paper show some of the difficulties encountered in the evaluation of seed treatments. Soil moisture affects the results very markedly, and so does the great difference in response to seed treatment between samples of disease-free wheat.

In the "dry" soil test the best treatment trebled the germination of the untreated seed, but the best treatment gave only 64.0% germination after incubation in dry soil compared with 95.0% for the untreated check sown in "moist" soil. Since germination in "dry" soil was negatively correlated with the amount of seed-coat injury, it must be concluded that a sound seed coat is a better protectant against seed-decaying organisms than the seed treatments tested.

Although formalin treatment often reduces seed germination under normal conditions, under "dry" conditions seed treated with formalin may germinate better than untreated seed (Table XI).

Since seed dressings are usually tested for effectiveness against smuts and seed-borne pathogens, preparations effective for controlling the "storage" molds infecting cereal seeds in "dry" soil and flax in "moist" soil may have escaped attention.

## Conclusions

Although cereal seeds sown in soil of subgermination moisture content do not germinate they can absorb water. The increased moisture content of the seed encourages infection by fungi. The invading fungi penetrate the seed and they, or the toxins produced by them, kill the embryo.

A "dry" soil, as here defined, approximates one-half the minimum moisture content (air-dry basis) required for germination. The "dry soil germination test" is defined as the germination under moist conditions after the seed has been sown in "dry" soil, and incubated for 9 days at 20° C.

The incubation period for the development of the fungi and death of the seed in "dry" soil varies inversely with the temperature, being 6 days at 20° C, and 14 or more days at 5° C.

Slightly saline "dry" soil increased, and strongly saline "dry" soil decreased, the subsequent germination of wheat seed.

Elite or Foundation seed and Commercial seed are equally susceptible to fungal infection.

Hull-less seeds are more susceptible to infection by fungi than seeds with hulls. The mean germination of all Elite and Foundation seed produced in 1949 and 1950 for wheat, rye, oats, and barley was 33, 50, 13, and 14% less in the "dry soil germination test" than when similar seed was sown directly in "moist" soil.

Damage to any part of the seed reduces subsequent germination in dry soil. Susceptibility of a seed lot was correlated to threshing injury of the seed coat over the embryo of wheat and to skinning or dehulling of oats and barley. Samples having 10 to 20% embryo-exposed seeds frequently showed 20 to 40% reduction in germination by the "dry soil germination test". Seed with natural injuries, such as frost damage, sprouting, or growth cracks in the endosperm, are more susceptible to fungal infection than undamaged seed.

In wheat the greater the weight per bushel, the greater the susceptibility to threshing injury. Similarly, straight grades are more susceptible than tough or damp grades.

The shape of the kernel affects its susceptibility to threshing injury. Durum wheat is more subject to seed injury than common wheat. Among the latter Redman and Selkirk are susceptible, and Lee and Thatcher moderately resistant to threshing injury.

Non-germinating seeds in "dry" soil were frequently infected with *Penicillium* spp. over the embryo. *Aspergillus*, *Rhizopus*, and *Cephalothecium* spp.

also caused embryo injury and death. These fungi, the "storage molds", indicate a similarity of conditions between seed incubated in "dry" soil and seed stored in a tough or damp condition.

When seeds naturally infected with *Helminthosporium* spp. were sown in "dry" soil, they were invaded by the storage molds which were antagonistic to *Helminthosporium* spp. The reduced germination was due to storage molds, not to the *Helminthosporium*.

Although treatment with New Improved Ceresan of all samples of Thatcher wheat, Victory oats, and Montcalm barley of the 1949 crop gave no increase in germination in "moist" soil over the untreated seed, the same samples sown in "dry" soil gave subsequent increases in germination averaging 13, 5, and 9%, respectively.

Soaking wheat for 4 hours in cold water tripled the subsequent germination, an increase which was almost equal to mercurial treatments, but no treatment gave complete control against fungal infection when the seed was sown in "dry" soil. Therefore, a sound seed coat is a better protectant against seed-decaying organisms than any treatment tested.

The potential value of a seed sample would be easier to appraise if, besides the "percentage germination", the "percentage of injured seeds" was known.

Threshing injury can be reduced by proper adjustments to the machine, and threshing when the grain is slightly tough.

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# SOME EFFECTS OF SMELTER POLLUTION NORTHEAST OF FALCONBRIDGE, ONTARIO[1]

EVILLE GORHAM AND ALAN G. GORDON

## Abstract

A study along a line NNE. from the metal smelter at Falconbridge, Ontario, reveals that strong sulphate accumulation in the surface soil occurs only within about one mile of the chimneys emitting sulphur dioxide pollution while effects upon the soil drainage waters are marked to a distance of nearly two miles, and still clearly evident 10 or more miles away. The number of species present in the flora declines sharply within about four miles of the smelter, but certain species (e.g. *Pinus strobus*, *Vaccinium myrtilloides*) disappear at much greater distances. Among the most tolerant species are *Acer rubrum*, *Quercus rubra*, *Sambucus pubens*, and *Polygonum cilinode*.

## Introduction

Air pollution and damage to vegetation by sulphur dioxide from metal smelters have been studied in detail at Trail in British Columbia (3), and to a lesser extent at Sudbury in Ontario (2, 4, 6). The present paper reports measurements of sulphate concentration in soils and pond waters, together with an examination of plant distribution, along a transect roughly NNE. from the Falconbridge smelter near Sudbury.

The Falconbridge smelter is about 14 miles NE. of that at Copper Cliff, and about 7 miles NNE. of the Coniston smelter. It is the smallest of the three in the Sudbury area; ore and concentrates charged ranged from 231,000 to 475,000 short tons annually between 1936 and 1946; in comparison the charges at Copper Cliff and Coniston ranged from 1,659,000 to 4,149,000 and 334,000 to 987,000 tons respectively (1). The reasons for choosing the Falconbridge area to examine were varied: firstly, there is a series of small kettle-hole ponds lying NNE., near to the NE. direction of the prevailing summer winds; secondly, the habitats in the direction of the prevailing wind are more uniform, and have been less disturbed by human settlement, than near the other smelters; and thirdly, any pollution emanating from the other smelters will generally be maximal at the beginning of the Falconbridge transect. This accessory pollution should also compensate (to an unknown degree) for the transect being slightly off the prevailing wind direction.

## Methods

During June 1959 a series of surface waters was collected from lakes and ponds at varying distances N. and NNE. of Falconbridge. In addition two small lakes were sampled near Emerald Lake, 35 miles to the NE., and on McLean Peninsula of Lake Temagami, 39 miles ENE. of Falconbridge; it is believed that at these distances pollution effects are negligible.

[1]Manuscript received January 22, 1960.
Contribution from the Botany Department, University of Toronto, and the Research Branch, Ontario Department of Lands and Forests.

In the same month plant communities were examined in the same general areas, those chosen being situated on sandy soils in the locations most exposed to air-borne pollution; valleys and protected slopes were avoided. Again sites near Emerald Lake and Lake Temagami were used as controls. The floristic study was conducted by laying out a 20-meter chain at random in each site, and recording the macrophyte species present less than 1 meter from either side of the chain. In addition some 10–20 minutes (depending on the nature of the cover) were spent in listing any accessory species observed in the same habitat near the chain.

Wherever the chains were placed for plant lists, two samples of the humus layer were also collected for measurement of soluble sulphate in the surface soil. Near the smelter such samples were difficult to find, since erosion (due to removal of the plant cover by pollution damage, and secondarily by fire) has eliminated the humus layer almost everywhere in the more exposed situations. This is reflected also in the lower ignition losses (mainly organic matter) of the humus layers near the smelter, which have been much contaminated by blown and drifting mineral soil exposed through erosion. Beyond 10 miles from the smelter ignition losses ranged from 54 to 85% dry weight, while within that distance the range was from 19 to 64%.

The soil samples were dried at just above 100° C, and aliquots were then shaken for 3 hours with 100 times their weight of distilled water. The extracts were filtered, and sulphate estimated by the technique of Mackereth (5), which was also employed on the pond waters. This technique includes any nitrate present along with the sulphate, but nitrates are likely to be of negligible importance in these soils and waters.

## Results

Some effects of smelter pollution upon the vegetation and its environment are shown in Table I and in Figs. 1 and 2. The first figure shows that the normal sulphate concentration in pond waters of the region is probably in the vicinity of 0.3 meq/l., to judge from the two samples collected at Emerald Lake and Lake Temagami, and also a sample from Wanapitei Lake, which drains extensive areas to the north and east of Sudbury and contained 0.34 meq of sulphate per liter on the date sampled. Owing to fall-out and oxidation of sulphur dioxide from the chimney stacks, sulphate levels rise gradually on approaching the smelter, to about 0.6 meq/l. at 3 miles distance, and then very sharply indeed nearer the smelter, concentrations of 1.2 to 1.5 meq/l. being recorded between 1.0 and 1.5 miles away. It may be added that two waters collected at 0.8 to 0.9 miles S. of the smelter exhibited sulphate levels of 3.5 and 2.8 meq/l., without apparently receiving other than aerial pollution. The point of inflection for the sulphate curve in Fig. 1 would appear to be at about 2 miles from the pollution source.

In the case of the humus layers, soluble sulphates varied from about 2 to 5 meq per 100 g dry weight of soil over the whole range of sites between 1 and 40 miles from the smelter, as shown in Fig. 2(*a*). Only at 1 mile or less were

TABLE I

Distribution of ground flora species in relation to air pollution by sulphur dioxide

| Species present in more than three of the 17 sites | Distance of site from smelter (miles) | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0.7 | 0.8 | 1.0 | 1.0 | 1.1 | 1.5 | 2.0 | 2.4 | 3.2 | 4.5 | 5.3 | 7.6 | 11 | 16 | 19 | 35 | 39 |
| *Sambucus pubens* | * | * | | * | | + | * | | | | * | | | | | | |
| *Polygonum cilinode* | * | + | | * | | | * | | | * | + | | * | | | | |
| *Comptonia peregrina* | | | * | | * | * | | * | | | * | | | | | | |
| *Epilobium angustifolium* | | | | * | | | * | * | + | + | + | + | * | | | | |
| *Salix humilis* | | | | + | * | * | * | * | + | + | * | * | * | + | | | |
| *Populus tremuloides* | | | | * | | | + | * | + | * | + | + | * | * | | | |
| *Acer saccharum* | | | | * | | + | + | * | * | | | | | | | * | |
| *Populus grandidentata* | | | | * | * | * | + | * | * | + | + | | | | | * | |
| *Smilacina racemosa* | | | | | | * | + | | * | + | | | | | | | |
| *Apocynum androsaemifolium* | | | | | | | | | * | * | * | + | | | | | |
| *Danthonia spicata* | | | | | | | | | | + | * | + | | | * | | |
| *Acer rubrum* | * | * | * | + | + | * | * | + | + | + | + | + | + | + | + | + | |
| *Quercus rubra* | * | * | * | + | + | + | + | + | | + | * | | | + | + | * | |
| *Diervilla lonicera* | | * | * | * | * | + | + | + | + | + | + | + | * | + | | + | + |
| *Lonicera canadensis* | | * | | * | | + | + | * | + | * | + | + | + | * | | + | + |
| *Aster macrophyllum* | | * | | + | | * | + | * | + | + | + | + | | + | + | + | + |
| *Maianthemum canadense* | | * | | * | | * | + | * | + | + | + | + | + | + | + | + | + |
| *Corylus cornuta* | | | + | + | + | + | + | + | + | + | + | * | | + | + | + | + |
| *Vaccinium angustifolium* | | | + | | * | | * | + | + | + | + | + | + | + | + | + | + |
| *Aralia nudicaulis* | | | * | | | + | * | | + | + | + | + | + | + | + | + | + |
| *Cornus canadensis* | | | | * | | | | | + | * | | + | | + | | | + |
| *Prunus pensylvanica* | | | | * | * | * | + | * | * | + | + | + | | + | | | * |
| *Betula papyrifera* | | | | + | | | | * | + | + | + | + | + | + | + | + | + |
| *Orysopsis asperifolia* | | | | | | + | + | + | + | + | * | | | | + | * | |
| *Acer spicatum* | | | | | | * | | | * | | * | + | + | + | | * | * |
| *Amelanchier sanguinea* | | | | | | * | | | | | | + | * | | + | | + |
| *Pteridium aquilinum* | | | | | | | * | + | * | + | + | * | | + | + | + | + |
| *Clintonia borealis* | | | | | | | | | * | * | * | | | + | + | + | + |
| *Lycopodium obscurum* | | | | | | | | | + | + | * | * | * | * | | | + |
| *Picea mariana* | | | | | | | | | | | | * | + | + | + | + | + |
| *Abies balsamea* | | | | | | | | | | | | * | + | + | + | | + |
| *Pyrus decora* | | | | | | | | | | | | * | + | | + | * | + |
| *Trientalis americana* | | | | | | | | | | | | + | + | + | + | + | + |
| *Linnaea borealis* | | | | | | | | | | | | + | + | + | + | * | + |
| *Pinus strobus* | | | | | | | | | | | | | | * | + | + | + |
| *Vaccinium myrtilloides* | | | | | | | | | | | | | | + | + | * | + |
| Total no. species per quadrat | 0 | 1 | 2 | 6 | 3 | 8 | 12 | 7 | 16 | 21 | 19 | 26 | 19 | 31 | 26 | 19 | 30 |
| Total no. species per site | 5 | 9 | 8 | 18 | 9 | 20 | 26 | 21 | 24 | 32 | 34 | 40 | 37 | 42 | 31 | 29 | 36 |

NOTE: + signifies presence in 20 m × 2 m quadrat, * signifies presence outside the quadrat.

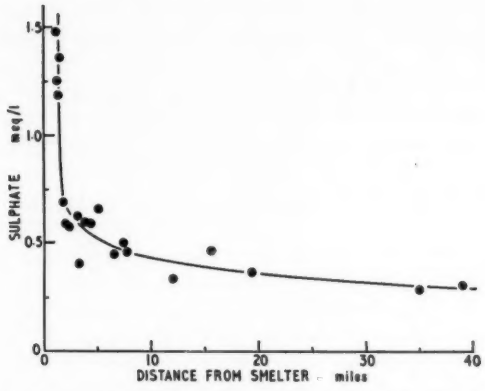


FIG. 1. Sulphate concentrations in surface waters at various distances NNE. and N. of the Falconbridge smelter (curve fitted freehand).

higher values recorded, ranging up to 20 meq per 100 g at the site nearest the pollution source, but still about 0.75 miles away. The secondary rise in sulphate beyond 10 miles distance, which coincides with a marked increase in soil ignition loss, suggests that the organic fraction is the important source of sulphate in these soils, and that a more valid expression of sulphate concentration might be given by expressing it per 100 g of ignition loss. This is done in Fig. 2(*b*), which reveals approximately the same picture as Fig. 2(*a*), except that the sulphate levels from 1 to 10 miles distance appear slightly higher than those beyond this limit. The soil extracts ranged in pH from 3.9 to 7.1, values below 4.9 were recorded only at 1 mile or less from the smelter, where however values up to the maximum were also observed.

As might be expected, the floristic studies showed a marked decrease in the number of species as the smelter was approached. In the case of the quadrats (20 m × 2 m), the more distant sites gave a range of 19 to 31 species, the number falling rapidly below the above minimum within 4 miles of the source of pollution, so that at less than 1 mile away only one species was present in one of the two random quadrats (Table I). The total species lists for each site gave a generally similar picture, with the distant sites ranging between 29 and 42 species, and those within about 4 miles of the smelter exhibiting a marked decline in numbers. In the two sites examined at less than a mile away, the widely scattered plants belonged to only 10 species.

It may be remarked that while species numbers remain above the minimum for the distant sites until within about 4 miles of the smelter, curves indicating the general trends appear to inflect at about 7 miles distance. Which distance should be taken as the true point of inflection may at present be regarded as a matter of choice. The numbers of species in the total lists are maximal at about 8 miles from the Falconbridge smelter. While the tendency to high numbers at intermediate distances may be accidental, the sites in this area

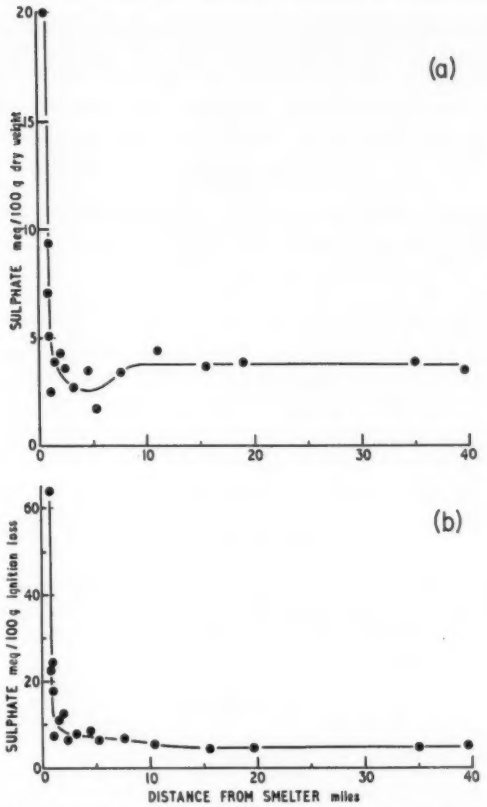


FIG. 2(*a*). Soluble sulphate in humus layers at various distances NNE. and N. of the Falconbridge smelter, expressed on dry weight basis (curve fitted freehand).
FIG. 2(*b*). Soluble sulphate in humus layers at various distances NNE. and N. of the Falconbridge smelter expressed on humus basis (curve fitted freehand).

appear to have been burned more frequently than those farther away from Falconbridge, so that the higher total numbers of species may equally well reflect a greater openness in the plant cover, with a consequent lessening of competition.

A detailed picture of the influence of air pollution upon the ground flora is also given by Table I, which lists the presence in the 17 sites of all species recorded in more than three of them (trees of more than 2 in. basal diameter being excluded). At the top of the list is a group of plants, most of which are not common in the more mature forests of the region, but are enabled by their relative tolerance of sulphur dioxide to colonize the ground once competition from normal forest species is reduced owing to air pollution. As might be expected, these are mainly seral species, and of these *Sambucus pubens* and *Polygonum cilinode* appear to be the most tolerant.

Following this group of plants is a series of species which are able to exist both in the normal mature forest and in more or less heavily polluted situations. *Acer rubrum* and *Quercus rubra* are the most characteristic of these, the first being almost ubiquitous. Reading down the list, species of lesser tolerance are encountered, until finally the most sensitive species appear. Among these last are *Vaccinium myrtilloides* and, unfortunately, *Pinus strobus*, a tree of great economic importance. The latter species was first observed at 16 miles along the transect NNE. of Falconbridge, but it may be noted that Linzon (4) was able to find a suitable plot for the study of this tree's response to air pollution at about 11 miles N. of this smelter, where however considerable damage was observed. At both 16 and 19 miles distance in the present study the white pines were commonly chlorotic.

Finally it may be worth remarking that the following species were recorded in three of the five sites more than 10 miles NNE. along the transect from the Falconbridge smelter: *Picea glauca*, *Viburnum cassinoides*, *Ribes glandulosa*, *Gaultheria procumbens*, *Polypodium virginianum*, and *Dicranum majus*. Ferns (three species, in four of the five sites) were observed only beyond 10 miles distance, but were nowhere abundant.

## Acknowledgments

We are much indebted to Mr. Keith Acheson, Regional Director of the Ontario Department of Lands and Forests, for kindly arranging the use of a helicopter; and to the pilot, Mr. R. F. Ross, for so readily placing us in so many difficult situations.

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# THE CHLOROPHYLL CONTENT OF SOME NATIVE AND MANAGED PLANT COMMUNITIES IN CENTRAL MINNESOTA[1]

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## Abstract

Concentration of chlorophyll per unit area of land surface was measured for six forests and 13 native and managed herbaceous stands in central Minnesota. Chlorophyll samples were extracted in acetone and in ethyl ether, and spectrophotometric analysis made of chlorophyll *a* and *b* content. Wet and ovendry weights of the aboveground crop were determined.

A highly significant correlation was found between the dry weight of the aboveground crop of the annual herbaceous stands and the chlorophyll content of these stands. All stable natural herbaceous stands had an approximately similar ratio of chlorophyll to the dry weight of aboveground parts.

Chlorophyll content in grams per square meter of land surface along an upland gradient from field crop through native communities of increasing age or successional development or both was *Zea mays* (2.7), *Soja max* (0.9), younger to older successional stages of old field (0.3 to 0.6), native prairie (0.7), xeric (more open) savanna (0.6), mesic (less open) savanna (1.0), conifer–hardwood forest (3.1). Chlorophyll content for a pond hydrosere from the *Nymphaea odorata* margin through *Carex lasiocarpa* mat to *Chamaedaphne calyculata* shrub and *Larix laricina* forest increased from 0.3 to 1.4 g/m². Values for a younger and older *Populus tremuloides* lowland grove were 1.7 and 5.9 and for a *Typha* marsh were 4.6 g/m².

These data showed a tendency for later successional stages to exceed earlier stages, for some lowland stands to exceed upland stands, for forest to exceed herbaceous communities on a given moisture level, and for field crops to exceed prairie and old field. The upland old field to forest sequence and the lowland *Nymphaea* to *Larix* sequence had similar chlorophyll contents for a given stage of successional development. There was a highly significant positive correlation between chlorophyll content and height of the arboreal stands, and a significant positive correlation between chlorophyll content and herbaceous stand height.

A significant difference in chlorophyll content was found between all stands with three or more sample plots, except those with closely correspondent mean values. The maximum difference between stands was twentyfold, which does not support previous studies which have suggested a similarity of chlorophyll content in diverse communities.

Chlorophyll concentrations and wet and dry weights per various plant parts are presented for the seven tree samples, and the presence of chlorophyll in other than leaf parts emphasized.


## Introduction

As part of an investigation of techniques for estimating the productivity of terrestrial vegetation, a study was made of the chlorophyll content of a diversity of native and managed plant communities to determine the magnitude of values and establish whether there were sufficient differences in magnitude to justify further sampling.

## Methods

### *Description of the Sample Area*

The sampled stands were located within the Cedar Creek Natural History Area (45° 24′ N., 93° 10′ W.) 48 km north of Minneapolis, Minnesota, on the

[1]Manuscript received January 15, 1960.
Contribution from the Department of Botany, University of Toronto, Toronto, Ontario.

adjacent farm of Alvar Peterson; and at Bunker Prairie, 5 km northwest of Johnsville, Minnesota. Each of these areas is a part of the Anoka Sand Plain discussed by Cooper (4). A description of some environmental and vegetational features of the Cedar Creek Area including information on temperature, precipitation, and solar radiation has been given in Bray *et al.* (2). Bunker Prairie is similar to the Cedar Creek Area in substrate and topography, although sand dune formation is more extensive and in some parts is still active. There is no evidence that Bunker Prairie has been plowed and the land has not been grazed recently. The area is still subject to fire, the most recent of which burned in spring of 1957 through a part of the prairie planted in pines.

*Description of Vegetation*

The dominant species of the 19 sampled stands are listed in Table I; complete presence lists for upland stands are presented in Table II and for lowland stands in Table III. Nomenclature in these tables and throughout the paper is after Gleason (8).

The upland stands included in Table II were separated from the lowland stands by their well-drained surfaces and by predominantly sand soils. The order of stands in this table is from cropland of *Zea mays* and *Soja max* through abandoned crop fields of increasing age and successional development (stands 4*a*, 4*b*, 4*c*, 3) to stable prairie (stand 12), to savanna of increasingly closed canopy (stands 11, 6), and to closed canopy forest (stand 2). The order is, thus, from a highly managed crop community through natural communities of increasing complexity and, in part, successional development. A straight-line successional sequence is not implied by the order of natural stands in Table II, although forest apparently replaces old field prairie over most of the area in absence of further disturbance. Certainly, the two savanna stands

TABLE I

Nature of the stands sampled

| Stand No. | Dominant species | Mean max. height, meters | Dry wt. 100 kg/ha | Chlorophyll, g/m² |
|---|---|---|---|---|
| 7*a* | *Zea mays* | 1.9 | 70 | 2.66 |
| 7*b* | *Soja max* | .5 | 16 | .91 |
| 4*a* | *Setaria glauca* | .4 | 19 | .30 |
| 4*b* | *Aristida basiramea* | .4 | 17 | .33 |
| 4*c* | *Sorghastrum nutans* | 1.4 | 27 | .37 |
| 3 | *Stipa spartea* | 1.3 | 35 | .59 |
| 12 | *Andropogon gerardi* | 1.5 | 27 | .74 |
| 11 | *Quercus macrocarpa* | 4.3 | — | .59 |
| 6 | *Quercus ellipsoidalis* | 4.0 | — | 1.02 |
| 2 | Conifer–hardwood forest | 13.1 | — | 3.08 |
| 10*a* | *Nymphaea odorata* | — | 11 | .35 |
| 10*b* | *Equisetum fluviatile* | — | 32 | .96 |
| 10*c* | *Carex lasiocarpa* | — | 48 | 1.27 |
| 10*d* | *Carex lasiocarpa* | — | 41 | 1.30 |
| 10*e* | *Chamaedaphne calyculata* | — | 81 | 1.00 |
| 8 | *Larix laricina* | 7.3 | — | 1.42 |
| 1 | *Populus tremuloides* | 4.0 | — | 1.68 |
| 5 | *Populus tremuloides* | 18.1 | — | 5.95 |
| 9 | *Typha latifolia-angustifolia* | 2.4 | 140 | 4.65 |

## TABLE II

Species composition of sampled upland stands and plots

| Species | Stand No. 7a | 7b | 4a | 4b | 4c | 3 | 12 | 11 | 6 | 2 |
|---|---|---|---|---|---|---|---|---|---|---|
| *Chenopodium album* | P | P | | | | * | P | * | | |
| *Polygonum convolvulus* | P | P | * | | | | | | | |
| *Setaria glauca* | P | P | D | | | * | | | | |
| *Zea mays* | D | | | | | | | | | |
| *Ambrosia trifida* | | P | | | | | | | | |
| *Soja max* | | D | | | | | | | | |
| *Ambrosia psilostachya* | | | P | P | P | P | P | P | P | |
| *Aristida basiramea* | | | P | D | P | P | | P | P | |
| *Asclepias tuberosa* | | | * | | * | | | | * | |
| *Cenchrus pauciflorus* | | | * | * | | | P | * | | |
| *Digitaria ischaemum* | | | * | | | | | | | |
| *Eragrostis spectabilis* | | | * | P | | | | * | * | |
| *Hedeoma hispida* | | | P | P | * | | | | | |
| *Lactuca canadensis* | | | * | | | | | | | |
| *Physalis virginiana* | | | * | | | | | * | P | |
| *Poa pratensis* | | | D | P | P | D | P | D | D | |
| *Polygonella articulata* | | | P | | | | | | | |
| *Prunus serotina* seedlings | | | | | | | | | | |
| *Cyperus filiculmis* | | | | * | * | * | P | * | | |
| *Erigeron strigosus* | | | | * | | | | | * | |
| *Euphorbia maculata* | | | | * | | | | | | |
| *Helianthus hirsutus* | | | | * | | | | | | |
| *Lathyrus ochroleucus* | | | | * | | | | | | |
| *Lespedeza capitata* | | | | P | P | * | * | * | * | |
| *Panicum lanuginosum* var. *fasciculatum* | | | | * | * | | | | | |
| *Panicum oligosanthes* | | | | P | * | P | * | * | P | |
| *Polygonum tenue* | | | | * | * | * | | * | | |
| *Quercus ellipsoidalis* seedlings | | | | * | | | | * | | * |
| *Verbascum thapsus* | | | | * | * | | | | | |
| *Agrostis hyemalis* | | | | | * | | | | | |
| *Andropogon gerardi* | | | | | * | * | D | * | D | |
| *Andropogon scoparius* | | | | | D | D | P | * | * | |
| *Antennaria plantaginifolia* | | | | | * | | | | * | |
| *Aster asureus* | | | | | * | | | | * | |
| *Carex pensylvanica* | | | | | * | | * | | | D |
| *Helianthus laetiflorus* | | | | | * | P | | * | P | |
| *Lathyrus venosus* | | | | | P | | * | | P | |
| *Lithospermum canescens* | | | | | * | * | | | * | |
| *Sorghastrum nutans* | | | | | D | * | * | D | * | |
| *Stipa spartea* | | | | | * | D | D | D | P | |
| *Amorpha canescens* | | | | | | * | | P | * | * |
| *Artemisia ludoviciana* | | | | | | * | P | * | * | |
| *Asclepias syriaca* | | | | | | * | | * | * | |
| *Aster ericoides* | | | | | | * | * | * | * | |
| *Bouteloua hirsuta* | | | | | | P | * | * | | |
| *Calamovilfa longifolia* | | | | | | * | | * | * | |
| *Campanula rotundifolia* | | | | | | * | | | | |
| *Carex muhlenbergii* | | | | | | * | | * | | |
| *Chrysopsis villosa* | | | | | | * | * | * | | |
| *Conyza canadensis* | | | | | | * | | | * | |
| *Coreopsis palmata* | | | | | | P | * | | * | |
| *Equisetum hiemale* var. *elatum* | | | | | | * | | | * | |
| *Graphalium obtusifolium* | | | | | | * | | | | |
| *Helianthemum bicknellii* | | | | | | * | | * | * | |
| *Koeleria cristata* | | | | | | P | * | * | | |
| *Liatris aspera* | | | | | | * | * | * | | |
| *Lithospermum caroliniense* | | | | | | P | * | * | | |
| *Mirabalis hirsuta* | | | | | | * | P | * | * | |
| *Mollugo verticillata* | | | | | | * | * | * | | |
| *Oenothera parviflora* | | | | | | * | | * | * | |
| *Panicum capillare* | | | | | | * | | * | | |
| *Polygala polygama* | | | | | | * | | * | | |
| *Potentilla arguta* | | | | | | * | * | * | * | |
| *Prunus pumila* | | | | | | * | * | | | |
| *Prunus virginiana* | | | | | | * | | * | | * |
| *Ranunculus rhomboideus* | | | | | | * | | * | | |
| *Rosa suffulta* | | | | | | P | P | * | P | |
| *Salix humilus* | | | | | | P | * | | * | |
| *Solidago nemoralis* | | | | | | P | * | * | * | |
| *Solidago rigida* | | | | | | * | P | P | * | |
| *Viola pedatifida* | | | | | | * | * | * | * | |
| *Anemone cylindrica* | | | | | | | * | | | |
| *Anemone patens* | | | | | | | * | | | |
| *Aristida longiseta* | | | | | | | * | | | |
| *Artemisia caudata* | | | | | | | * | | | |
| *Aster ciliolatus* | | | | | | | * | | | |
| *Bouteloua curtipendula* | | | | | | | * | | | |
| *Celastrus scandens* | | | | | | | * | | | |
| *Cycloloma atriplicifolium* | | | | | | | P | | | |
| *Cyperus schweinitzii* | | | | | | | * | * | | |
| *Elymus canadensis* | | | | | | | * | | | |

TABLE II (*Concluded*)

Species composition of sampled upland stands and plots (*Concluded*)

| Species | Stand No. 7a | 7b | 4a | 4b | 4c | 3 | 12 | 11 | 6 | 2 |
|---|---|---|---|---|---|---|---|---|---|---|
| *Helianthus maximiliani* | | | | | | | * | | | |
| *Helianthus tuberosus* | | | | | | | P | | | |
| *Liatris punctata* | | | | | | | * | | | |
| *Panicum virgatum* | | | | | | | * | * | | |
| *Petalostemum candidum* | | | | | | | * | | * | |
| *Petalostemum purpureum* | | | | | | | * | * | | |
| *Rhus radicans* | | | | | | | * | | | * |
| *Smilacina stellata* | | | | | | | * | | | * |
| *Solidago graminifolia* | | | | | | | * | * | | |
| *Solidago missouriensis* | | | | | | | P | * | * | |
| *Solidago speciosa* | | | | | | | * | | | |
| *Spirea alba* | | | | | | | * | | | |
| *Sporobolus cryptandrus* | | | | | | | * | | | |
| *Delphinium virescens* | | | | | | | | * | | |
| *Euphorbia glyptosperma* | | | | | | | | * | | |
| *Fraxinus pennsylvanica* | | | | | | | | * | | |
| *Penstemon grandiflorus* | | | | | | | | * | | |
| *Quercus borealis* | | | | | | | | * | | |
| *Quercus borealis* seedlings | | | | | | | | * | | |
| *Quercus ellipsoidalis* | | | | | | | | D | D | D |
| *Quercus macrocarpa* | | | | | | | | D | * | * |
| *Quercus macrocarpa* seedlings | | | | | | | | * | * | P |
| *Rhus glabra* | | | | | | | | * | * | |
| *Rudbeckia hirta* | | | | | | | | * | | |
| *Tradescantia occidentalis* | | | | | | | | * | | |
| *Tragopogon dubius* | | | | | | | | * | | |
| *Antennaria neglecta* | | | | | | | | | * | |
| *Artemisia frigida* | | | | | | | | | * | |
| *Bromus kalmii* | | | | | | | | | * | |
| *Ceanothus americanus* | | | | | | | | | * | |
| *Corylus americana* | | | | | | | | | * | D |
| *Euphorbia corollata* | | | | | | | | | * | |
| *Fragaria virginiana* | | | | | | | | | * | * |
| *Galium boreale* | | | | | | | | | * | * |
| *Lupinus perennis* | | | | | | | | | * | |
| *Maianthemum canadense* var. *interius* | | | | | | | | | * | P |
| *Monarda fistulosa* | | | | | | | | | * | |
| *Phlox pilosa* | | | | | | | | | * | |
| *Rubus hispidus* | | | | | | | | | * | |
| *Scutellaria leonardi* | | | | | | | | | * | |
| *Sporobolus heterolepis* | | | | | | | | | * | |
| *Vicia americana* | | | | | | | | | * | |
| *Acer rubrum* | | | | | | | | | | * |
| *Acer rubrum* seedling | | | | | | | | | | P |
| *Amphicarpa bracteata* | | | | | | | | | | P |
| *Aquilegia canadensis* | | | | | | | | | | * |
| *Aster macrophyllus* | | | | | | | | | | * |
| *Gaultheria procumbens* | | | | | | | | | | P |
| *Oryzopsis asperifolia* | | | | | | | | | | * |
| *Parthenocissus vitacea* | | | | | | | | | | P |
| *Pinus banksiana* | | | | | | | | | | * |
| *Pinus strobus* | | | | | | | | | | * |
| *Pinus strobus* seedling | | | | | | | | | | * |
| *Populus grandidentata* | | | | | | | | | | * |
| *Pteridium aquilinum* var. *latiusculum* | | | | | | | | | | * |
| *Pyrola asarifolia* | | | | | | | | | | * |
| *Pyrola secunda* | | | | | | | | | | * |
| *Rubus strigosus* | | | | | | | | | | * |
| *Vaccinium angustifolium* | | | | | | | | | | D |
| *Veronicastrum virginicum* | | | | | | | | | | * |
| *Viola incognita* | | | | | | | | | | * |

NOTE: D = Dominant or codominant, P = present in one or more of the sample plots, * = present in the stand but not present in a sample plot.

are not a stage between old field prairie and forest, although they are slowly converting to forest at present. The old field stands are arranged, however, in one of the secondary successional patterns for the region. The order of stands in Table II is a relational order in the sense that stands which are similar in species composition are in close proximity in the table and least related stands are at the greatest distance. From stand 7 to 4*a* there is a decreasing height and weight on a landscape area basis of the dominant species and from 4*b* to 2 an increasing height and weight.

*Zea mays* (stand 7*a*) and *Soja max* (stand 7*b*) have been in crop and pasture rotation for the past several years and have been under cultivation since before 1900 (Pierce (12)). Stand 7*a* was fertilized with nitrogen and stand 7*b* was manured immediately before the 1958 growing season. The rows in both fields were aligned in an east–west direction. Stand 7*a* is on low-lying ground and has a highly organic sand soil; it receives some runoff water and is adjacent at its lower end with a sedge meadow. The *Zea mays* crop in 7*a* was planted for cattle fodder and there is a dense distribution of stalks (4.3 stalks per meter). The rows were a mean distance apart of .88 m and the mean height per plant to the top of the tassel was 1.9 m. Stand 7*b* is on a gently sloping hill and is well drained. The soil is not highly organic. *Soja max* in 7*b* had a distribution of 2.3 stalks per meter and the rows were a mean distance of .94 m apart. The mean height per plant was .64 m. There were several areas of the field in which there was a sparse distribution of *Soja* stems; some of these areas were included in the randomly laid samples.

Stands 4*a*, 4*b*, and 4*c* correspond to stands 57-9, 57-10, and 57-11 of Bray *et al.* (2) and represent three areas of an old field whose age since abandonment is not known, although vegetational appearance indicates it was abandoned in the late 1930's or early 1940's, more probably the latter. The order of these stands in Table II is from annual stages with *Setaria glauca* (stand 4*a*) and *Aristida basiramea* (stand 4*b*) to the perennial stage dominated by *Sorghastrum nutans* and *Andropogon scoparius* (stand 4*c*). Of the annual stages, *Setaria* is the earlier and it often occurs in the Cedar Creek Area as a weedy cover in fields of *Zea mays*, *Soja max*, and *Secale cereale*. All of these three native grass communities are within a small area of less than an acre; their present composition appears to be related to the closeness to source of dissemules in that stand 4*c* is at the edge of the old field adjacent to a small roadside prairie and thus has closer access to prairie seeds and migrating rhizomes than stands 4*a* or 4*b* which are farther into the field.

Stands 4*a*, 4*b*, and 4*c* are of more recent origin than stand 3 which was abandoned in fall of 1927, in which year a potato crop was harvested. Stand 3 was never completely plowed or grubbed (removal of roots of oak grubs) before it was abandoned; the crops were planted in plowed furrows (Alvar Peterson, personal communication, information from John Mickelson, the last owner). Stand 3 resembles a mature native prairie and is dominated by *Stipa spartea*, *Andropogon scoparius*, and *Poa pratensis*. There are few weeds or pioneer native prairie species present and the grass and forb cover is dense and continuous over most of the area. It is characteristic of sand areas in central Minnesota and Wisconsin which have been grazed or plowed that they return to prairie within 20 to 30 years after abandonment. Thompson (14) noted that on sand in central Wisconsin, prairie was well established 15 years after abandonment and that by 35 years, forest invasion was predominant and quoted Shimek's observations that complete prairie re-establishment in Iowa required 30 years. The Goldschmidt prairie at Riverside, Wisconsin, was abandoned in the mid 1930's after having been grazed for several decades

(F. J. Goldschmidt, personal communication) and in 1958 it resembled the native prairies of the railroad right of ways in the area.

Stands 12*a*, 12*b*, and 12*c* represent three separate areas of the Bunker Prairie. Stand 12*a* is in the tree plantation burned in spring of 1957 and is dominated by *Andropogon gerardi* and *Stipa spartea*. Its prairie cover had decreased in vigor from shading by pines and is now recovering following the destruction of the pines in the fire. Stand 12*b*, dominated by *Andropogon gerardi*, is on a part of the prairie not recently burned. Stand 12*c* in which *Andropogon gerardi* and *Elymus canadensis* predominate is along a railroad right of way and charcoal remnants indicate it has burned within the past 3 years. The order of stands from 12*a* to 12*c* is towards increasing stem density and vigor of growth. The plants in 12*c* were especially luxuriant and there was the greatest production of flowering stalks in this area. The stimulus of fire in promoting flowering in a prairie which is similar in composition to 12*c* was noted by Dix and Butler (6).

Stand 11 is an excellent example of *Quercus* savanna vegetation. It occupies a series of gently to steeply rolling sand dunes, a few of which are still active. There are low moist prairies and poorly drained swales at the base of some of the dunes. The area has been pastured, but never plowed, probably because of the steepness of some of the dune hills and the apparent low fertility of the sand soil. The barb wire fence surrounding the stand is now very rusted and it is unlikely that the area has been disturbed, other than by fire, since the early 1930's. An indication of the apparent low soil fertility in the stand is the stunted condition of the scattered *Quercus ellipsoidalis* and *Quercus macrocarpa* which are the dominant tree species. Most of these trees were less than 7 m in height and many were presumably over 100 years in age. The *Quercus macrocarpa* sampled in stand 11 had 86 growth rings to within 0.01 m of the center, at which point it was hollow from decay. This tree was 4.3 m in height. The ground flora in stand 11 contains several xeric prairie species not encountered elsewhere in the study including *Delphinium virescens*, *Euphorbia glyptosperma*, *Penstemon grandiflorus*, and *Tradescantia occidentalis*, and the proportion of such xeric grasses as *Bouteloua hirsuta* and *Koeleria cristata* was higher than in the other upland stands. The dominant understory species were *Stipa spartea*, *Sorghastrum nutans*, and *Poa pratensis*.

Stand 6 is a *Quercus* savanna which contains the same tree dominants as stand 11, but these trees are taller and more densely distributed, and there is a greater density of saplings and seedlings. Stand 6 is more mesic in its understory composition than stand 11 or than the prairie areas in stands 3, 4, and 12 as shown by the presence of such species as *Ceanothus americanus*, *Corylus americana*, *Fragaria virginiana*, *Galium boreale*, *Monarda fistulosa*, and *Phlox pilosa* each of which reach peak presence in the Mesic or Mesic–Wet prairie intervals of Curtis (5). The more mesic aspect of stand 6 may be partly the result of topography since the area is fairly low, abutting on a *Typha* marsh at one corner, and is without active dunes. The greater density of trees and shrubs also contributes to this condition. There are many shrubs (mostly

*Corylus americana*) and shrubby thickets in the stand which indicates the area has not been heavily burned recently.

Stand 2 is an upland forest similar to those described within the conifer–hardwood forest of central and northern Wisconsin by Brown and Curtis (3). The canopy of stand 2 is almost closed and it represents an early intermediate stage in which *Pinus banksiana*, *Quercus ellipsoidalis*, and *Quercus macrocarpa* are being replaced by more shade tolerant species including *Acer rubrum*, *Pinus strobus*, and *Quercus borealis*. The *Pinus banksiana* trees are in the "broom" stage in which their canopy is very narrow and there is little annual growth increment. The stand has been grazed and burned in the past and its tree composition is non-homogeneous. There are a few recent disturbance gaps which have filled with *Populus grandidentata*, and parts of the stand are sufficiently open to allow the survival of stunted prairie plants including *Amorpha canescens*. *Quercus ellipsoidalis* is the dominant tree species with *Carex pensylvanica* and *Corylus americana* comprising much of the understory along with scattered *Vaccinium angustifolium*.

The lowland stands in Table III occur on muck or highly organic soils and receive runoff water with the exception of stand 1 which is in a draw and is well watered but has a sandy soil. Stands 10*a* through 10*e* were sampled at equal intervals back from the *Nymphaea* zone at the lake margin of Beckman Lake, a small shallow pond, the middle of which is now almost constricted by vegetation encroachment. The depth from the soil surface to the water level is shown for the Beckman Lake stands in Table IV. The order of stands follows a typical hydrophytic successional gradient for the area, from *Nymphaea odorata* in stand 10*a* to a mixture of *Nymphaea* and *Equisetum fluviatile* in stand 10*b* to a mingling of these species with *Carex lasiocarpa* (stand 10*c*) to an area dominated by *Carex lasiocarpa* and *Calamagrostis canadensis* (stand 10*d*) to a shrubby zone with *Chamaedaphne calyculata* and *Spiraea tomentosa* (stand 10*e*). One of the possible further points for this successional development is a *Larix* swamp and stand 8 was included at the conclusion of the 10*a* to 10*e* sequence for this reason. Stand 8 occurred within 0.4 km of stand 10 and was part of a much larger lowland area which is separated from Beckman Lake by a low sand ridge. It is probable that the lowland area around stand 8 was open water in early postglacial times and has since been filled with peat through stages in hydrach succession. There is a narrow *Larix* area at both ends of Beckman Lake similar in compostion to stand 8 although its successional relationship with the lake margin sequence in stand 10 is not clear. The *Larix* areas have little or no *Chamaedaphne* and have a ground cover with an abundance of *Sphagnum* spp. and *Carex lasiocarpa*, whereas the *Chamaedaphne* shrub in 10*e* has little *Sphagnum* or *Carex lasiocarpa*. There is the possibility that *Larix* and *Chamaedaphne* are alternate successional routes or that a disturbance factor, as fire, favors the replacement of *Larix* by *Chamaedaphne* or prevents the invasion of *Chamaedaphne* by *Larix* once the shrub stage is established. Both stands 8 and 10*e* can be regarded, then, as developmental stages from the *Carex lasiocarpa* mat, with a possibility that *Larix* (stand 8) has developed in the absence of disturbance.

TABLE III

Species composition of sampled lowland stands and plots

| Species | Stand No. | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 10a | 10b | 10c | 10d | 10e | 8 | 1 | 5 | 9 |
| *Utricularia* sp. | * | | | | | | | | |
| *Nymphaea odorata* | D | D | P | | | | | | |
| *Equisetum fluviatile* | P | D | * | P | | | | | |
| *Potamogeton natans* | | * | | | | | | | |
| *Sparganium chlorocarpum* | | * | | | | | | | |
| *Sagittaria latifolia* | | P | * | P | * | | | | * |
| *Typha latifolia* | | * | * | P | | | | | |
| *Calamagrostis canadensis* | | * | * | P | P | * | | | |
| *Dulichium arundinaceum* | | * | P | P | | * | | | |
| *Iris versicolor* | | | * | | | | | | |
| *Lycopus uniflorus* | | | * | | | | | | |
| *Lysimachia terrestris* | | | * | | | | | | |
| *Triadenum virginicum* | | | * | | | | | | |
| *Cicuta bulbifera* | | | * | * | * | | | | |
| *Galium trifidum* | | | P | P | | | | | |
| *Potentilla palustris* | | | * | * | | | | | |
| *Thelypteris palustris* | | | * | * | | * | | | |
| *Carex lasiocarpa* | | | D | D | P | P | | | |
| *Alnus rugosa* | | | | * | | | | | |
| *Andromeda glaucophylla* | | | | * | | | | | |
| *Campanula aparinoides* | | | | * | | | | | |
| *Epilobium leptophyllum* | | | | * | | | | | |
| *Erechtites hieracifolia* | | | | * | | | | | |
| *Eupatorium perfoliatum* | | | | * | | | | | |
| *Osmunda regalis* | | | | * | | | | | |
| *Salix petiolaris* | | | | * | | | | | |
| *Scutellaria epilobiifolia* | | | | * | | | | | |
| *Viola pallens* | | | | * | | | | | |
| *Aronia melanocarpa* | | | | * | | * | | | |
| *Chamaedaphne calyculata* | | | | * | D | P | | | |
| *Polygonum sagittatum* | | | | * | * | * | | | |
| *Salix discolor* | | | | * | * | * | | | |
| *Scirpus cyperinus* | | | | * | * | * | | | |
| *Spirea tomentosa* | | | | * | P | * | | | |
| *Vaccinium oxycoccos* | | | | * | P | | | | |
| *Aster puniceus* | | | | | * | | | | |
| *Epilobium coloratum* | | | | | * | | | | |
| *Phragmites communis* | | | | | * | | | | |
| *Rhus vernix* | | | | | * | * | | | |
| *Spirea alba* | | | | | * | | | | |
| *Betula pumila* | | | | | | * | | | |
| *Carex brunnescens* | | | | | | * | | | |
| *Dryopteris austriaca* | | | | | | * | | | |
| *Dryopteris cristata* | | | | | | * | | | |
| *Ilex verticillata* | | | | | | * | | | |
| *Larix laricina* | | | | | | * | | | |
| *Ledum groenlandicum* | | | | | | * | | | |
| *Osmunda cinnamomea* | | | | | | * | | | |
| *Rubus strigosus* | | | | | | * | | | |
| *Sphagnum* spp. | | | | | | D | | | |
| *Vaccinium angustifolium* | | | | | | * | | | |
| *Corylus americana* | | | | | | | D | | |
| *Populus tremuloides* | | | | | | | D | D | |
| *Rubus allegheniensis* | | | | | | | P | | |
| *Acer rubrum* seedling | | | | | | | | P | |
| *Alnus rugosa* | | | | | | | | * | |
| *Aralia nudicaulis* | | | | | | | | P | |
| *Arisaema triphyllum* | | | | | | | | * | |
| *Athyrium felix-femina* | | | | | | | | D | |
| *Clintonia borealis* | | | | | | | | P | |
| *Ilex verticillata* | | | | | | | | * | |
| *Lactuca biennis* | | | | | | | | * | |
| *Maianthemum canadense* var. *interius* | | | | | | | | * | |
| *Osmunda claytoniana* | | | | | | | | * | |
| *Parthenocissus vitacea* | | | | | | | | * | |
| *Prenanthes alba* | | | | | | | | * | |
| *Prunus serotina* seedling | | | | | | | | * | |
| *Quercus borealis* seedling | | | | | | | | * | |
| *Rhus radicans* var. *rydbergii* | | | | | | | | * | |
| *Viola incognita* | | | | | | | | P | |
| *Typha latifolia-angustifolia* | | | | | | | | | D |
| *Lemna* spp. | | | | | | | | | * |

Stands 1 and 5 represent a younger and older *Populus tremuloides* grove. In both stands this species is the sole tree species in the canopy. These groves are common throughout the area and are usually succeeded by various combinations of species, including *Acer rubrum*, *Betula lutea*, *Betula papyrifera*,

TABLE IV

Chlorophyll content, replicate subsamples
Values in grams per sample

| Stand No. | Sample No. 1 | Sample No. 2 |
|---|---|---|
| 3(*d*) | .4 | .6 |
| 3(*e*) | .4 | .4 |
| 6 lvs. (A) | 10.0 | 10.7 |
| 6 lvs. (B) | 10.3 | 10.1 |
| 8 understory | .5 | .8 |
| 9(*b*) | 3.2 | 3.3 |
| 9(*c*) | 6.8 | 6.4 |
| 11 lvs. | 11.7 | 11.7 |

*Fraxinus nigra*, *Fraxinus pennsylvanica*, *Ostrya virginiana*, *Prunus serotina*, *Quercus borealis*, and *Ulmus americana*, to form a lowland hardwood forest. Both *Populus* stands are well watered; stand 5 is particularly moist with a rich organic soil and a lush understory cover with *Athyrium felix-femina* as the dominant species.

Stand 9 includes the same area as stands 57-4 and 57-12 of Bray *et al.* (2), and is dominated by a vigorous hybrid complex between *Typha latifolia* and *Typha angustifolia*. The only other flowering plants occasionally present are *Lemna* spp. and *Sagittaria latifolia*. The *Typha* swamp lies between a road and the margin of Fish Lake and its dense growth and high productivity (see Bray *et al.*) are perhaps enhanced by the washing of nutrients from the limestone gravel of the road, and by wind-drifted fish carcasses from the winter die-offs. The mean height of these plants in 1957 was 2.2 m while the mean height in 1958 was 2.4 m.

*Field Sampling Techniques*

*Herbaceous and Shrubby Plots*

All aboveground plant material in the herbaceous and shrubby plots, excluding duff and litter, was clipped and taken to the Cedar Creek Laboratory for subsampling. A square quadrat .91 m on a side was used for the predominantly low grass cover in stands 3, 4, and 12 and for the understory plots in stands 1, 2, 5, 6, 8, and 11. A circular quadrat 0.61 m in radius was used in stands 9 and 10 in which there was a predominance of large stalked plants. The circular quadrat was defined by a wire 0.61 m in length which was attached to a central stake. During clipping, the plant material immediately on either side of the edge of both the square and circular quadrats was carefully separated and approximately half the material pushed to the outside of the quadrat and the other half collected so as to reduce the error of edge effects. The quadrat shape for the field crops in stand 7 was a rectangle, one-half the distance from a sampled row to its neighbor rows in width and an average of 3 m in length.

The herbaceous material was taken to the laboratory immediately upon collection. The usual time between clipping a quadrat and collecting a chlorophyll subsample was from 15 to 30 minutes. The chlorophyll subsamples were

taken from the entire collected field material which included both living and dead stems. This was done in order to lessen the time between the clipping of the quadrat and the subsample collection. If the living and dead materials had been separated in many of the grassy herbaceous quadrats, several hours would have elapsed before the subsamples could have been taken and an appreciable chlorophyll degradation might have occurred. The subsamples were made with a heavy scissors by bunching together a sheaf of plant material and cutting a small cross section from this sheaf. This cross section was usually 0.005 m in length, and between 15 and 30 such cross sections were taken for each subsample. The resulting subsample was weighed and placed in a small polyethylene bag which was tightly tied and placed in the subfreezing compartment of the refrigerator. Thus, within an hour and often within 30 minutes from the time of field collection, the chlorophyll subsample had been placed in the cold and dark where chlorophyll degradation would occur very slowly. At the same time that the chlorophyll subsample was weighed, the quadrat sample was weighed so that the final computation of total chlorophyll per quadrat was based on a ratio of a determined weight of chlorophyll in a known weight of fresh subsample against a calculated weight of chlorophyll for a known fresh sample weight.

The chlorophyll subsamples were transported from the refrigerator at Cedar Creek to a refrigerator in the laboratory at Minneapolis in evening of the day of collection, and in all instances but one, the chlorophyll extraction was made the following day. The samples upon removal from Cedar Creek were placed in a large polyethylene bag and surrounded by ice cubes. This bag was securely tied and placed in several heavy paper bags which were also sealed. There was little melting of the ice cubes on the 1-hour trip from Cedar Creek to Minneapolis, and the large plastic bag when opened next morning would often have no melt water. In one instance, the sampling of Bunker Prairie, the chlorophyll subsamples were made in Minneapolis. For this sampling, the material clipped from each quadrat was placed in a polyethylene bag and this bag placed in a larger one containing ice cubes; these bags were wrapped in a tarpaulin. The ice cubes needed for the six quadrats in stand 12 were transported to Bunker Prairie in their original heavy paper containers as dispensed from the ice machine.

In most of the graminoid herbaceous quadrats, a single subsample or a series of two or four replicate subsamples of the entire plant material would be taken. In other quadrats, however, separate chlorophyll subsamples would be made for various parts of the plant, depending on the ease with which each of these parts could be sampled. One result of this breakdown into separate parts was that tissue with high chlorophyll content would not be mixed in an unrepresentative proportion with tissue of low chlorophyll content. In *Zea mays*, for example, the leaves were separated from the ears and stem which have a much lower content on a weight basis. The usual procedure was to include plant material in a single subsample if it seemed homogenous in chlorophyll content, but to separate it if otherwise.

*Arboreal Plots*

Seven trees were sampled for chlorophyll content; information on their size and weight is included in Table VII. Before cutting a tree, two separate measurements were made of its canopy extent. The first measurement taken was the area of actual canopy perimeter from a series of diameters, each of which was estimated by standing directly beneath the farthest extent of the canopy and sighting upward. The resultant series of diameters were averaged and a mean circular area computed. The canopy shapes of each of the seven sampled trees was approximately circular. The second measurement was termed the "effective" canopy and estimated the mean sky area which the tree had effectively to itself. If two canopies were not in contact, the terminus of the effective canopy diameter was extended to a point which was at one-half the distance between the canopies. If tree limbs were in contact, the effective canopy diameter extended to midway the distance of the overlap area. The sum of the percentage actual canopies could, thus, be less than 100 in a savanna or open forest and greater than 100 in a densely canopied forest, whereas the sum of the percentage effective canopies must always be 100. All estimates of chlorophyll content per unit area were made, therefore, in relation to the effective canopy measurements. In the savanna stands it was impractical to measure half the distance to adjacent canopies and measurement of the percentage canopy cover for the stand as a whole was made by a series of line intercepts. Total chlorophyll per unit area was then calculated by a ratio between the amount measured per the actual canopy area of the sample tree and the amount per the total canopy area of the stand. Understory quadrats for closed canopy forests were laid directly beneath the canopy of the tree or trees sampled in the stand. For savanna stands, chlorophyll subsamples were taken from quadrats placed beneath the tree canopy and also in the open. The chlorophyll content of these subsamples was then prorated in figuring total understory content according to the percentage of open and canopied areas in the stand.

Each tree upon being cut was carried from the stand in sections and taken to the laboratory where chlorophyll subsamples were made. Leaf subsamples were taken by collecting a set of leaves and cutting a series of cross sections through each set with a scissors. These leaf subsamples were processed as previously described for the non-forest herbaceous material. To obtain the wet weight of the leaves of the angiosperm trees sampled, the entire leaf crop was stripped by hand and weighed as it was removed. Since the leaves remained attached to the twigs and trunk sections until picked, there was little evidence of wilting by the time the crop was harvested, although it is likely that water loss occurred which was not replaced by water from the trunk. As a precaution to prevent drying, the windows of the laboratory were kept closed during the leaf collections and, by chance, all but one of the tree sample days were humid. For *Pinus banksiana* and *Larix laricina*, it was not possible to separate the leaves in the available time, and a series of subsamples were separated into needles and branches for parts of the tree and the remainder weighed en masse. The subsample weights were then used to determine the weight

of needles and branches for the canopy as a whole. In one instance (*Populus tremuloides* stand 1), leaf subsamples of known area were clipped with a punch and the chlorophyll content of the clipped disks compared with the total leaf surface area of the canopy. This area was determined by counting squares on graph paper for a subsample of known wet weight. The ratio between leaf surface area (one surface only) and canopy area as projected on the ground for this tree was 3.30.

Chlorophyll subsamples of the smaller twigs were made by clipping a series of narrow cross sections which were representative of the various twig diameters. These samples, including the wood, were later extracted in entirety since chlorophyll was plainly visible in the wood (presumably in the xylem parenchyma) of the younger twigs of some species, including *Quercus macrocarpa* and *Corylus americana*. These subsamples were made on a wet weight basis and processed as were the leaf subsamples. For larger branches and the trunk, chlorophyll subsamples were based on a known portion of the total trunk and branch length. Circular strips of bark 0.0254 m in width were peeled at regular intervals along these stems and then processed as described. With three workers, the leaf canopy of the larger angiosperm trees could be stripped in 1 to 3 hours with the subsequent stem dismemberment occupying up to an additional 3 hours. The result of applying the above methods would be a number of chlorophyll subsamples on a wet weight basis of the leaves and smaller branches and a correspondent wet weight measure of these parts plus subsamples of known lengths of the larger stems with measurements of their lengths.

*Harvest Samples*

For each of the understory plots and trees in the study, all or a substantial portion of the collected material was oven-dried at 70° C. The entire stems of smaller trees, and cross sections of 0.0254 m in width taken at regular intervals from the larger tree branches and trunks, were used in the drying subsample. Leaf and small twig samples were dried from 2 to 4 days and larger stem samples dried a minimum of 2 weeks. The aboveground dry weight increments for stands sampled in 1957 which were resampled in 1958 (Nos. 4*a*, 4*b*, 4*c*, 7*a*, and 9) were comparable in relative magnitude although the samples were not taken at exactly the same period. Wet and dry weight measurements are included in Tables I, V, and VI on an area basis and in Table VII on an individual basis for the sampled trees.

*Laboratory Analytic Techniques*

Methods of chlorophyll extraction and spectrophotometric analysis were based on techniques of Arnon (1), Zscheile and Comar (16), and Pearson and Lawrence (11). All herbaceous and tree leaf material was extracted in acetone, while most bark and wood tissue and some leaf material in which tannins and other substances stained the acetone extract were further separated in ethyl ether. In these extractions, one sample at a time was processed. This sample was removed from the refrigerator and placed in a Waring blender to which was added acetone and a pinch of $CaCO_3$; the blender was

TABLE V

Chlorophyll content and standing crop, upland plots

| Stand No. | Plot No. | Date | Plant part | Wet wt. 100 kg/ha | Dry wt. 100 kg/ha | Chlorophyll *a*, g/m² | Chlorophyll *b*, g/m² |
|---|---|---|---|---|---|---|---|
| 7*a* | 1 | 8/8 | Shoots | 393 | 46 | 1.58 | 1.15 |
| 7*a* | 2 | 9/3 | Shoots | 525 | 94 | 1.44 | 1.15 |
| 7*b* | 1 | 8/8 | Shoots | 82 | 15 | .69 | .18 |
| 7*b* | 2 | 8/27 | Shoots | 61 | 15 | .24 | .21 |
| 7*b* | 3 | 8/27 | Shoots | 97 | 19 | .49 | .43 |
| 4*a* | 1 | 7/18 | Shoots | 21 | 12 | .12 | .12 |
| 4*a* | 2 | 9/3 | Shoots | 49 | 26 | .19 | .17 |
| 4*b* | 1 | 7/18 | Shoots | 23 | 14 | .23 | .22 |
| 4*b* | 2 | 8/30 | Shoots | 52 | 20 | .12 | .09 |
| 4*c* | 1 | 7/18 | Shoots | 24 | 14 | .20 | .19 |
| 4*c* | 2 | 8/30 | Shoots | 109 | 41 | .16 | .20 |
| 3 | 1 | 7/ 8 | Shoots | 52 | 28 | .41 | .24 |
| 3 | 2 | 7/18 | Shoots | 50 | 30 | .42 | .39 |
| 3 | 3 | 8/27 | Shoots | 55 | 35 | .20 | .20 |
| 3 | 4 | 8/27 | Shoots | 55 | 33 | .29 | .32 |
| 3 | 5 | 8/27 | Shoots | 74 | 49 | .24 | .25 |
| 12*a* | 1 | 9/7 | Shoots | 68 | 24 | .35 | .46 |
| 12*a* | 2 | 9/7 | Shoots | 39 | 18 | .17 | .23 |
| 12*b* | 1 | 9/7 | Shoots | 75 | 34 | .35 | .37 |
| 12*b* | 2 | 9/7 | Shoots | 47 | 22 | .29 | .37 |
| 12*c* | 1 | 9/7 | Shoots | 63 | 29 | .31 | .41 |
| 12*c* | 2 | 9/7 | Shoots | 89 | 37 | .50 | .64 |
| 11 | 1 | 8/30 | Tree (Q.m.) | | | .12 | .11 |
| 11 | 1 | 8/30 | Understory | 38 | 22 | .19 | .17 |
| 6 | 1 | 8/2 | Tree (Q.e.) | | | .31 | .20 |
| 6 | 1 | 8/8 | Understory | 56 | 28 | .25 | .26 |
| 2*a* | 1 | 7/16 | Tree (P.b.) | | | 1.36 | 1.00 |
| 2*a* | 1 | 7/16 | Understory | 37 | 7 | .30 | .20 |
| 2*b* | 2 | 7/12 | Tree (Q.m.) | | | 1.93 | 1.26 |
| 2*b* | 2 | 7/12 | Understory | 8 | 2 | .08 | .04 |

TABLE VI

Chlorophyll content and standing crop, lowland plots

| Stand No. | Plot No. | Date | Depth water table, cm | Plant part | Wet wt. 100 kg/ha | Dry wt. 100 kg/ha | Chlorophyll *a*, g/m² | Chlorophyll *b*, g/m² |
|---|---|---|---|---|---|---|---|---|
| 10*a* | 1 | 8/20 | +56 | Shoots | 69 | 12 | .17 | .12 |
| 10*a* | 2 | 9/10 | +58 | Shoots | 58 | 11 | .25 | .16 |
| 10*b* | 1 | 8/20 | +43 | Shoots | 178 | 36 | .70 | .53 |
| 10*b* | 2 | 9/10 | +38 | Shoots | 133 | 28 | .40 | .30 |
| 10*c* | 1 | 8/20 | + 9 | Shoots | 296 | 60 | .96 | .84 |
| 10*c* | 2 | 9/10 | +12 | Shoots | 136 | 37 | .38 | .38 |
| 10*d* | 1 | 8/20 | 0 | Shoots | 114 | 46 | .81 | .75 |
| 10*d* | 2 | 9/10 | 0 | Shoots | 84 | 37 | .56 | .49 |
| 10*e* | 1 | 8/20 | − 8 | Shoots | 156 | 78 | .55 | .52 |
| 10*e* | 2 | 9/10 | − 5 | Shoots | 141 | 84 | .50 | .43 |
| 8 | 1 | 8/14 | −10 | Tree (L.l.) | | | .37 | .26 |
| 8 | 1 | 8/14 | −10 | Understory | 425 | 77 | .44 | .35 |
| 1 | 1 | 7/12 | | Tree (P.t.) | — | — | .82 | .61 |
| 1 | 1 | 7/12 | | Understory | 103 | 49 | .14 | .11 |
| 5 | 1 | 7/24 | | Tree (P.t.) | | | 2.98 | 1.90 |
| 5 | 1 | 7/24 | | Understory | 65 | 14 | .68 | .39 |
| 9 | 1 | 8/20 | | Shoots | 502 | 127 | 2.78 | 2.55 |
| 9 | 2 | 9/3 | | Shoots | 478 | 136 | 1.44 | 1.34 |
| 9 | 3 | 9/3 | | Shoots | 549 | 156 | 3.14 | 2.70 |

TABLE VII

Chlorophyll composition per plant, trees

| Stand No. | Species | Plant part | Wet wt., g | Dry wt., g | Chlorophyll *a*, g | Chlorophyll *b*, g |
|---|---|---|---|---|---|---|
| 1 | *Populus tremuloides* | Blades | 160 | 62 | .310 | .233 |
| | Age = 13, height = 3.94 m, | Petioles | 17 | 7 | .001 | .001 |
| | D.B.H. = .015 m, canopy diam. = .70 m | Small branches | — | 581 | .001 | .001 |
| | | Large branches, trunk | — | | .046 | .045 |
| | | Plant, total | — | 650 | .358 | .280 |
| 8 | *Larix laricina* | Needles | 856 | 438 | .784 | .524 |
| | Age = 31, height = 7.26 m, | Branches | — | 2,646 | .082 | .072 |
| | D.B.H. = .099 m, canopy diam. = 1.75 m | Trunk | — | 12,864 | .022 | .023 |
| | | Plant, total | — | 15,948 | .888 | .619 |
| 2*a* | *Quercus macrocarpa* | Leaves | 3,382 | 1,173 | 4.010 | 2.633 |
| | Age = 54, height = 11.07 m, | Stem bark | — | 26,603 | .061 | .037 |
| | D.B.H. = .105 m, canopy diam. = 2.25 m | Stem wood | — | | .003 | .003 |
| | | Plant, total | — | 27,776 | 4.074 | 2.673 |
| 2*b* | *Pinus banksiana* | 1-Year needles | 3,520 | 1,466 | 1.391 | 1.234 |
| | Age = 60, height = 15.32 m, | >1-Year needles | 4,480 | 1,865 | 3.464 | 2.327 |
| | D.B.H. = .170 m, canopy diam. = 1.65 m | 1-Year branches | 3,575 | 2,337 | .033 | .025 |
| | | >1-Year branches | 2,515 | 1,644 | .052 | .046 |
| | | Trunk | — | 111,660 | .041 | .037 |
| | | Epiphytes | — | — | .003 | .004 |
| | | Plant, total | | 118,972 | 4.984 | 3.673 |
| 6 | *Quercus ellipsoidalis* | Leaves | 3,642 | 1,603 | 6.342 | 3.940 |
| | Age = 25, height = 4.04 m, | Branches | — | 1,237 | .259 | .218 |
| | D.B.H. = .067 m + .036 m, | Trunk | — | 16,107 | .000 | .000 |
| | canopy diam. = 2.55 m | Plant, total | | 18,947 | 6.601 | 4.158 |
| 11 | *Quercus macrocarpa* | Leaves | 9,264 | 4,198 | 5.970 | 5.759 |
| | Age = 86 +, height = 4.27 m, | Twigs <1/2 in. | 7,881 | 4,674 | .225 | .202 |
| | D.B.H. = .178, canopy diam. = 3.52 m | Twigs 1/3–2/3 in. | 6,253 | 4,463 | .071 | .068 |
| | | Branches >2/3 in. | 30,217 | 16,485 | .096 | .106 |
| | | Trunk | — | 32,864 | .122 | .122 |
| | | Plant, total | | 62,684 | 6.484 | 6.257 |
| 5 | *Populus tremuloides* | Blades | 8,877 | 3,158 | 20.253 | 12.620 |
| | Age = 30, height = 15.92 m, | Petioles | 1,922 | | .177 | .1 3 |
| | D.B.H. = .185 m, canopy diam. = 3.17 m | Twigs <1/4 in. | 10,619 | 5,317 | .861 | .702 |
| | | Twigs 1/4 – 1 in. | 12,382 | 6,401 | .671 | .406 |
| | | Branches and trunk | — | 89,250 | .298 | .273 |
| | | Plant, total | | 100,968 | 22.260 | 14.144 |

run at high speed for 3 to 5 minutes and the extract then removed into a beaker. Before the extract was poured from the blender, a path was cleared with a scraper down one side of the blender glass to prevent green plant material from being washed in an unextracted condition into the beaker. The sides of the blender glass and the blender glass lid were then scraped and another quantity of acetone was added by washing down the sides of the blender glass. A further extraction was then made and this process repeated until the material was judged to be completely extracted. Two criteria were used in this judgment: the lack of greenish color in the remaining plant material and the lack of color in the acetone following extraction. Most leaf blades and petioles were reduced to a white or grey sludge and the bark and wood material to a brown fibrous remainder at the time the acetone extract was considered to be colorless. The amount of acetone needed to complete extraction varied widely with species. Grasses often required 10 to 20 times the amount needed for angiosperm tree leaves or crop plant leaves. *Aristida basiramea* and *Poa pratensis* were the most difficult to extract and in several samples it was necessary to grind the material in sand before extraction was complete. The minimum acetone required was 69 cc (for a 2.9-g *Typha* leaf sample), the mean amount was 335 cc, and the maximum was 1197 cc (for an 8.2 g *Stipa spartea – Poa pratensis* sample).

When acetone extraction was complete, a portion of the extract was centrifuged at 5 *g* for approximately 5 minutes which was in all cases of sufficient time to clear the supernatant. The supernatant was then analyzed in a Beckman spectrophotometer model B at wave lengths of 6450 and 6630 Ångströms. Three readings were taken at each wave length using a sensitivity of 2 in both cases. Chlorophyll concentrations were ascertained using the absorption coefficients of Arnon.

Most bark and wood material and some leaf samples, after extraction in acetone, were further extracted in ethyl ether. After 50 cc ether and an equal amount of acetone extract were added to a separatory funnel, the funnel was tilted gently to mix the fluids, but never inverted. This mixture was washed with 1000 cc or more of water by initially adding the water slowly from a microwashbottle and later more rapidly from a beaker. These precautions prevented emulsifying the chlorophyll and were continued until there was no motion in the drained water which indicated the presence of acetone. The remaining chlorophyll–ether solution was usually of a volume of 1 or 2 cc less than the original 50 cc ether which indicated there had been a slight loss by evaporation. The chlorophyll–ether solution was analyzed at 6425 and 6600 Ångströms with three readings per band and a sensitivity of 2, and chlorophyll concentrations were calculated with the Zscheile and Comar absorption coefficients.

By processing one sample at a time, there was a minimum chlorophyll loss from light; the extract was protected from effects of plant acids by the $CaCO_3$. The sample had been protected from heat and drying by the manner of collection and storage. The reliability of the acetone and ethyl ether spectrophotometric methods has been found to decrease when either chlorophyll

*a* or *b* becomes three to five times more concentrated than the other (15). Such ratios did not occur in material analyzed in the present study; the greatest *a*:*b* ratio was 3.0 (for south-facing *Populus tremuloides* leaves at the top of the tree). Table IV lists the chlorophyll content per sample of a series of replicate subsamples. Each subsample represents a different collection of plant material and the presented results give the consistency of the total operation of sampling the plant and extracting its chlorophyll. Two samples in this table show percentage differences in relation to the mean value of between 20 and 25%, the remainder range from 0 to 5%. The four leaf subsamples from stand 6 are within a mean of 2% variation from the mean chlorophyll content. There is, therefore, a fairly high reproducibility in the sampling procedure for a given plot and especially so for the higher chlorophyll contents.

The accuracy of the absolute magnitude of chlorophyll content is more difficult to assess. There was presumably a slight water loss from the subsample between cutting and subsequent weighing; the subsample would, then, weigh less than it should in relation to the sample weight and the calculated chlorophyll value would be higher than the actual content. The evaporation of water from the leaves of tree species before they could be picked and weighed would lessen their weight in relation to the subsample weight and, thus, lower the chlorophyll estimate. In the chlorophyll extraction, incompleteness of extraction into the acetone would decrease the chlorophyll estimate. The extraction of most of the leaf samples in acetone without a further separation into ethyl ether may have resulted in a slight proportion of tannins or other organic stains in the extract of those samples which contained dead plant material. Since tannin absorbs light, although in low proportion, at 6450 and 6630 Å, the result of tannin staining would be increased absorption at these bands and an enlarged chlorophyll estimate. This enlargement would be greatest for chlorophyll *b* since the tannin absorption spectrum decreases from 6630 to 6450 Å. Most of the chlorophyll *a*:*b* ratios in the present study are less than 2.0 which is below the range of from 2.5 to 3.5 summarized in Rabinowitch (13) for land plants. The low *a*:*b* ratios for the grass species plots in Table V which contained dead plant material may be, therefore, an indication of tannin staining. The chlorophyll ratios for tree leaf and non-graminoid herb samples are probably valid, however, since the ratio for *Populus tremuloides* leaves with acetone extraction of 1.6 (stand 5) is not appreciably different from the ratio for the same species in ether of 1.4 (stand 1). Chlorophyll ratios for *Populus tremuloides* leaves with ether extraction from Pearson and Lawrence (11) show a range of from 1.3 to 1.9. The lower *a*:*b* ratios in the tree leaf samples may be the result of sampling an entire plant canopy including both sun and shade leaves with the lower *a*:*b* ratio of the shade leaves reducing the ratio for the canopy as a whole. The presence of chlorophyll decomposition products, especially of pheophytin *a*, would have enlarged the absorption coefficient mainly in the chlorophyll *a* region and thus have overestimated the actual chlorophyll content. These decomposition products occur most frequently in dead plant material and would

have been most prominent in the grass samples. That such decomposition products may be of little significance is indicated by the low $a:b$ ratios in these samples.

## Results and Discussion

The results of the chlorophyll and harvest sampling are shown on a plot basis in Tables V and VI and for summarized stands in Table I. No estimates are available for underground production, but the aboveground dry weights of the sampled herbaceous stands represent an approximate annual net increment of the aerial plant portion, at least in the annual species. The values derived from the herbaceous plots are here considered as standards for comparison with chlorophyll content. Figure 1 shows the distribution of chlorophyll in relation to dry weight for these stands. A clearly linear pattern of increase is evident. The one value which is divergent from the trend is that for *Soja max* (stand 7*b*) and this stand, of all the herbaceous stands studied, had the least closed cover, the rows were distant in relation to the spread of the individual plants, and cultivation had fairly effectively decreased the weed crop. The chlorophyll content of 7*b* may be high, therefore, in relation to the dry weight increment, as might be expected from the greater sky space available to each individual plant. The trend in Fig. 1 was tested for degree of correlation after both the chlorophyll and dry weight values were transformed to a log basis. This transformation was made to normalize the distribution which is skewed by the presence of two high values of chlorophyll and dry weight. The resulting value of $r$ is +.89 and the probability of

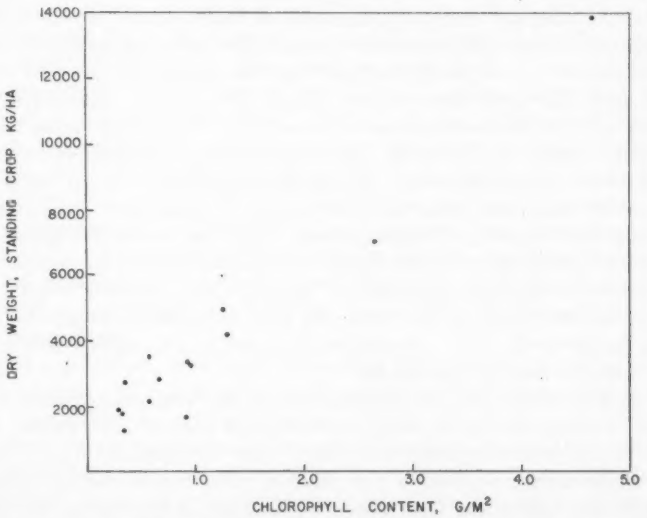


FIG. 1. The relationship between the dry weight of the standing crop of annual herbaceous communities and their chlorophyll content.

obtaining such a value by chance is $<.001$. The more productive stands judged by dry weight of crop are, therefore, also the more productive as judged by chlorophyll, which might be expected. If this correlation is valid for net production, including production of underground material, then the use of chlorophyll content as a productivity index for herbaceous vegetation is feasible. Whether a similar correlation exists for arboreal vegetation remains to be established.

The ratios between chlorophyll content and dry weight are fairly constant for the two sets of natural herbaceous stands (1.58, 1.94, 1.37 for the old field successional stands; 3.18, 3.00, 2.64, 3.17 for the annual hydrosere stands). The lower ratio of the woody hydrosere stand diverges in the expected direction. The stable prairie (stand 12) has a ratio of 2.74 which is comparable with the ratios for the herbaceous part of the hydrosere, and, therefore, all the stable natural herbaceous stands have an approximately similar percentage chlorophyll per unit of dry weight of aboveground parts.

The upland stands in Table I show a clearly defined trend in chlorophyll content along the vegetational gradient from cropland to forest presented in Table II. Maximum chlorophyll occurs in the *Zea mays* field and in the conifer–hardwood forest. The high values of the two field crops are probably determined, in part, by the fertilizer which both these crops have received. Minimum chlorophyll occurs in the *Setaria glauca* section of the recently abandoned old field, which is the earliest pioneer stage of the natural upland communities studied. The old field stands have not been recently burned and their lower chlorophyll content compared with the stable prairie (stand 12) which has been burned may be related to the immobilization of nutrients in the litter layer of the old field and a consequent decreased soil nutrient content. The gradual increase in chlorophyll through the perennial stage of the recent old field (stand 4*c*), the stable old field, and the prairie and savanna stands is readily seen. The only non-uniform value in this trend is that for stand 11, the more open of the two savanna stands, which has a lower value than that for prairie (stand 12) or stable old field (stand 3). A possible explanation for this lower value is that stand 11 was the most xeric of the upland stands as indicated by its species composition, the areas of sparseness in its grass cover, and the stunted condition of its scattered trees. The presence of trees in stand 11 does not compensate for the more xeric ground vegetation which is lower in chlorophyll, as shown in Table V, than the more densely sodded prairie cover in stands 3 and 12. The four old field successional stages in Table I (stands 4*a*–4*b*–4*c*–3) show a consistent increase in chlorophyll content from more pioneer to less pioneer stands.

Among the lowland stands, the hydrophytic successional sequence in Table I demonstrates a similar increase in chlorophyll content from earlier to later stages. If the sequence from open water with *Nymphaea* to *Larix laricina* forest is considered, neglecting the possibly disturbance-induced stage with *Chamaedaphne calyculata*, the chlorophyll increase is consistent. There is not a sufficient number of samples to state whether the *Chamaedaphne* stage could be expected to always show a lower chlorophyll content than the *Carex*

*lasiocarpa* meadow, but there seems little doubt of the trend of increasing chlorophyll with advancing vegetation development. Of the remaining lowland stands, the younger *Populus tremuloides* grove shows a lower value than the older grove, as could be expected, while there is no successional series or vegetation gradient with which to compare the value for *Typha* (stand 9).

A comparison among both upland and lowland stands shows a tendency for later successional stages to exceed earlier stages, for some lowland stands to exceed upland stands, for forest to exceed herbaceous communities on a given moisture level, and for field crops, probably because of addition of fertilizer, to exceed prairie and old field. The old field to forest upland sequence and the *Nymphaea* to *Larix* lowland sequence show a noteworthy similarity in chlorophyll content for a given stage. Both stand 4*a* and 10*a*, for example, at the pioneer end of these gradients have between 0.3 and 0.4 g/m², while the more advanced stages for both sequences are above 1.0 g/m².

The statistical significance of the differences in chlorophyll concentration for those stands in Tables V and VI which have three or more plot measurements can be assessed by using a small sample test for the significance of differences between means. In stand 4, all three developmental stages were considered jointly, and in stand 10, the three more advanced stages only (10*c*, 10*d*, 10*e*) were included. Each eligible stand was tested against every other, using a "*t*" test with $N_1 + N_2$ degrees of freedom, and the probability that the means were derived from the same population was calculated. The results of this test showed stand 9 to have a mean chlorophyll content which was significantly higher than that for stands 10, 7, 12, 3, or 4. The mean content of stands 10 and 7 was not different, but stand 10 was significantly higher than stands 12, 3, and 4. Stand 7 was significantly higher than stands 3 and 4, but was not so with stand 12. Stand 12 was not higher than stand 3, but was significantly higher than stand 4, and stand 3 was significantly higher than stand 4. Thus, for all tested stands except those with closely correspondent mean contents (10–7; 7–12; 12–3) the within stand plot variation was not sufficiently great to obscure the between stand differences. There were no forest stands included in this comparison since none of these stands contained a sample of three or more trees. In stand 2, however, there were two trees sampled and there is a close correspondence, as shown in Table V, between the total chlorophyll per unit area of the *Pinus banksiana* sample (2.86 g/m²) and that for the *Quercus macrocarpa* sample (3.31 g/m²) although the amount in the understory was less, and in the canopy was greater, for *Quercus macrocarpa*.

There is a difference of almost twentyfold between the stands of Table I with the highest and lowest chlorophyll contents. A difference of this degree has not been shown by other terrestrial studies and both Gessner (7) and Odum (9) have suggested that chlorophyll contents on an area basis may tend to be similar in diverse communities. The possibility of an overestimation of chlorophyll content for the grass samples of the present study has been noted, but correction of this error would only increase the maximum difference among the sampled stands. Of the herbaceous stands, both *Typha* hybrid

(stand 9) and *Setaria glauca* (stand 4*a*) are monocots and were sampled by the same extraction process and with the same time interval from field clipping to the taking of chlorophyll subsamples and the weighing of the fresh material. The sixteenfold difference in chlorophyll between these stands is a reasonable divergence considering the appearance of the two communities: *Setaria* is less than .5 m in height, *Typha* is over 2.0 m; there is a much greater density of *Typha* stem, a fourteenfold difference in fresh weight and a sevenfold difference in dry weight.

Further evidence for a diversity of chlorophyll content on a land area basis with differing types of plant community is shown in the increasing content of chlorophyll with increasing height of the community. The existence of this correlation was suggested by D. B. Lawrence (personal communication) for all communities with comparable life forms. To test this hypothesis, the data were separated into two series, one for primarily arboreal stands and the other for herbaceous stands. There was a highly significant positive correlation ($r = +.90$; $P = <.01$) of chlorophyll content with height in the arboreal series and a significant positive correlation ($r = +.79$; $P = <.05$) of chlorophyll content with height in the herbaceous series. Within the arboreal series, there were chlorophyll values from one stand of 3.31 g/m² for *Quercus macrocarpa* (height 11.1 m) and of 2.86 g/m² for *Pinus banksiana* (height 15.3 m). The deviation of these values from the demonstrated positive correlation may reflect the influence of individual species and of environmental heterogeneity.

Previous studies for terrestrial communities of absolute chlorophyll magnitude by Gessner (7) in Switzerland show values of 1.0 g/m² for a meadow dominated by *Dactylis glomerata* and *Galium mollugo* and values calculated from the literature of 1.34 g/m² for a *Fagus sylvatica* forest and of 0.96 g/m² for a *Betula* forest. Odum *et al.* (10) determined a content of 3 g/m² for a montane rainforest in the Luquillo Mountains of Puerto Rico.

Information on chlorophyll content per various plant parts and on the wet and dry weights of these parts is included in Table VII for the seven tree samples. The importance of chlorophyll in non-leaf tissue is evident from the presented values, with a maximum content of 14.6% in the young *Populus tremuloides* stem. The proportion of stem chlorophyll is less for the older sampled *Populus* compared with the younger plant, and there is proportionally more chlorophyll in the stem of the open-grown *Quercus macrocarpa* (stand 11) than in the closed-grown tree (stand 2). Pearson and Lawrence (11) found chlorophyll in bark of *Populus tremuloides*, and noted that the absolute amount of bark chlorophyll was higher in spring before leaves reach maximum size than later in summer. The participation of stem chlorophyll in starch production was demonstrated by Pearson and Lawrence for *Populus tremuloides* bark. The relative contribution of tree stem photosynthesis to total plant production is largely unknown.

There is a decrease in $a:b$ ratios for the data in Table VII from leaf material through twigs and small branches to larger branches and trunk. Almost all trunk samples show a ratio of 1.0. This trend is probably related to the influence of increasingly thick bark layers in shading the chloroplasts.

Chlorophyll weights in relation to dry and wet leaf weights are highest in the two *Populus* samples with values of 1.05 (dry) and .31 (wet) for stand 5 and of .79 (dry) and .31 (wet) for stand 1. The two conifer species have the lowest ratios with values of .28 and .14 for *Larix laricina* and .18 and .07 for 1-year-old needles of *Pinus banksiana*. The *Pinus* needles older than 1 year have ratios of .31 and .13. There is an appreciable decrease in these ratios between the forest-grown *Quercus macrocarpa* (.57 dry; .20 wet) and the open-grown tree (.28 dry; .13 wet) which reflects the increased chlorophyll content of shade leaves.

## Acknowledgments

Grateful acknowledgment is made to Dr. D. B. Lawrence, administrator of the ecosystem project of which the present study is a part, to the Louis W. and Maud Hill Family Foundation for financial assistance, and to Mr. Alvar Peterson for permission to sample his field crops. I am indebted to Messrs. A. Rogosin and V. Heig and Dr. L. C. Pearson for their assistance in the field and laboratory sampling, to Drs. Norman Good and L. C. Pearson for advice in the laboratory techniques for chlorophyll extraction, to Dr. J. W. Moore for aid in plant species identification, and to Mrs. Norah J. Nolan for dating the sampled trees. This work was pursued, in part, at the Cedar Creek Natural History Area, a research facility administered by the Minnesota Academy of Science and the University of Minnesota through Graduate School Dean T. C. Blegen and Prof. A. N. Wilcox, Director.

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# CHROMOSOME NUMBERS IN SOME MOSSES FROM QUEBEC[1]

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## Abstract

The results of chromosome studies of five species of mosses from the Mont Tremblant area of Quebec are presented. The chromosome numbers of two species, *Plagiothecium roeseanum* (Hampe) B.S.G., with the number $n = 20$, and *Polytrichum ohioense* Ren. & Card., with the number $n = 14$, are reported for the first time. Both species are established tetraploids with no evidence of chromosome irregularities in meiosis. Chromosome numbers reported for the other three species are as follows: *Dicranum rugosum* (Hoffm.) Brid., $n = 12$; *Dicranum scoparium* Hedw., $n = 12$; and *Plagiothecium denticulatum* Hedw., $n = 20$. The population of *D. rugosum* that was studied did not possess small precociously separating bivalents as have been reported for European populations. The close resemblance of the chromosome complements of *P. denticulatum* and *P. roeseanum* is in line with the close taxonomic relationship between the two species. Cytotaxonomic implications are discussed under each species.


## Introduction

Almost no cytological studies have been made of the mosses of Eastern Canada. Anderson and Bryan (1958) reported the chromosome numbers for five species, two from Quebec, *Dicranum flagellare* Hedw. and *Grimmia alpicola* Hedw., and three from Nova Scotia, *Barbula fallax* Hedw., *Heterophyllium haldanianum* (Grev.) Kindb., and *Pogonatum pensilvanicum* (Hedw.) Paris. This is much in contrast with Western Canada, where Anderson and Crum (1959) studied the chromosomes in 146 populations that embraced 62 species, mainly from the Canadian Rocky Mountains. It seems worthwhile, therefore, to record the results of studies made on five species of mosses, which were gathered in the vicinity of Mont Tremblant, near Saint Jovite, in the Laurentian Mountains of Quebec, in June of 1959.

## Materials and Methods

Chromosome studies were made on spore mother cells at meiosis. Living plants with developing sporophytes were collected, sealed in plastic bags, and shipped to the botanical laboratories of Duke University, where the investigations were carried out. A cold storage pretreatment was used, which consisted of storing the plants in a refrigerator at approximately 2° C. Details of this pretreatment have already been described (Anderson and Crum 1959). The cytological techniques employed were essentially the same as those which were described at length by Steere, Anderson, and Bryan (1954). Briefly, they consisted of squeezing out the contents of young moss capsules in a drop of Carnoy's solution (3:1) on a clean slide, freeing the spore mother cells of the columella and other possible extraneous tissues, allowing the fixative to

[1]Manuscript received January 19, 1960.
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evaporate almost completely, and adding a drop of acetic orcein (synthetic aceto-orcein saturated in 45% acetic acid and filtered). After a cover glass was added, pressure was applied in order to spread out the chromosomes, to flatten the division figures, and to squeeze out oil bodies or other optically troublesome cytoplasmic inclusions. The cover glass was sealed with vaseline and the spore mother cells were studied immediately.

A voucher specimen of each population studied has been deposited in the herbarium of Duke University. The results of the investigations will be described and discussed under the heading of each species.

## Results and Discussion

*Dicranum rugosum* (Hoffm.) Brid., $n = 12$ (Figs. 1–2)

Twelve large bivalents were observed at MI in sporocytes of this large handsome species. Good preparations were difficult to obtain, a fact that has been noted previously in other species of *Dicranum* (Bryan 1956; Anderson and Bryan 1958), because the chromosomes are sticky and bivalents tend to clump together in groups. It was found that chromosomes could be counted more easily at late MI and during AI. Figure 1 was drawn from a late metaphase I figure in which two bivalents had already separated. The respective half-bivalents have been indicated with arrows. An anaphase figure is depicted in Fig. 2, in which two groups of 12 half-bivalents each can be counted. Note in each figure that there is one large heterochromosome, the half-bivalents of which are especially evident in Fig. 2. Similar heterochromosomes have been described before in other species of *Dicranum* and Yano (1951*a*) has postulated a role in sex determination for the heterochromosome.

Holmen (1958) investigated the chromosomes of *D. rugosum* in Danish material in which he also found 12 large bivalents, but in addition reported a small *m*-bivalent. We were unable to find any evidence for the presence of a small bivalent in Canadian material, however. The existence of small or diminutive bivalents (also variously called *m*-bivalents and accessory chromosomes) is widespread in mosses and their presence or absence has been demonstrated to be characteristic of certain species of some genera and of certain intraspecific races in others (see, e.g., Bryan 1955; Anderson and Crum 1959). Interpretations must be made cautiously, however, because the diminutive bivalents in some species stain faintly and are difficult to demonstrate. Also there is some evidence that the staining qualities of the small bivalents may vary according to the condition of the material (Holmen 1958; Steere, Anderson, and Bryan 1954; Anderson and Crum 1959).

Yano (1951*b*) reported only 11 bivalents in Japanese populations of *D. rugosum* and found no evidence of a small bivalent. Yano's counts were based on somatic divisions in gametophytic plants, however, and there is still some question as to whether the small or diminutive bivalents can be demonstrated in somatic divisions. Bryan (1955) has discussed this point at some length in her studies of the chromosomes of *Sphagnum*.

*Dicranum scoparium* Hedw., $n = 12$ (Figs. 3–5)

This exceedingly variable and widely distributed species has been studied cytologically by several investigators, but there is disagreement concerning the chromosome number. As pointed out above, the chromosomes of *Dicranum* are notoriously sticky and are not infrequently difficult to interpret. Utilizing a slight modification in technique suggested by Mrs. Ardith Brask Johnsen, we were able to obtain very satisfactory preparations of *D. scoparium*. The modification involved nothing more than elimination of heat following the application of aceto-orcein. As noticed by Mrs. Johnsen, we found that heating preparations tended to increase stickiness of the chromosomes, which makes them more cohesive and more difficult to spread apart. By avoiding this step in the preparation of slides we noticed remarkable improvement in separation of chromosomes, especially at first meiotic metaphase.

A side view of first metaphase is shown in Fig. 3. The chromosomes are well spread and are less condensed than usual. The large heterochromosome, characteristic of the genus, is shown in about the center of the figure and a conspicuous satellite arm is visible. The heterochromosome could be traced through meiosis and is shown in the premetaphase group of the second meiotic division in Fig. 5 (in the lower group). Half-bivalents of the heterochromosome are shown in Fig. 4, which is a side view of AI. Previous investigators have not described a satellite chromosome in *D. scoparium* or any other species of the genus.

Japanese investigators have consistently reported the chromosome number in *Dicranum* as $n = 11$ (Shimotomai and Koyama 1932; Yano 1951*b*, 1957*b*), while American and European workers have uniformly interpreted the basic number as $n = 12$ (Bryan 1956; Anderson and Bryan 1958; Holmen 1958; Anderson and Crum 1959; Steere 1954). Both sets of investigators have studied *D. majus* Sm., *D. scoparium*, and *D. rugosum*, each with the number $n = 11$ in Japan, and $n = 12$ (with or without small bivalents) in Europe and North America. It seems highly improbable that these differences could be real and therefore must be due to differences in interpretation. As already pointed out, all of the counts from Japan were based on somatic divisions in gametophytic tissues while the American and European counts were made from meiotic divisions, but the reasons for such consistent disagreement are not evident.

Bryologists have in recent years split off several segregate genera from *Dicranum*, namely *Arctoa*, *Kiaeria*, and *Orthodicranum*. Unfortunately no counts are available yet for any of the species comprising *Arctoa*, which can be supported as a genus on rather strong taxonomic grounds, but species in *Kiaeria*, *Orthodicranum*, and *Dicranum* (sensu lato) appear to have the same basic chromosome number. Cytological evidence for maintaining or abolishing the latter genera is therefore inconclusive.

*Plagiothecium denticulatum* Hedw., $n = 20$ (Fig. 6)

This species, almost worldwide in its distribution, is one of the most variable and puzzling in all the Musci. Countless synonyms, segregate species, sub-

FIGS. 1–2. *Dicranum rugosum* (Hoffm.) Brid., $n = 12$; Fig. 1, polar view of late MI, showing 10 bivalents and four half-bivalents (at the end of an arrow); Fig. 2, polar view of early AI, showing 20 half-bivalents and two bivalents. ×1450

FIGS. 3–5. *Dicranum scoparium* Hedw., $n = 12$; Fig. 3, polar view of MI, notice the large chromosome with a satellite and another chromosome, smaller in size, with a satellite; Fig. 4, polar view of AI, showing 24 half-bivalents, note the two large half-bivalents; Fig. 5, polar view of late AI, note the large chromosome with the satellite. ×1450

FIG. 6. *Plagiothecium denticulatum* Hedw., $n = 20$; polar view of MI, showing 20 bivalents. ×1450

FIGS. 7–9. *Plagiothecium roeseanum* (Hampe) B.S.G., $n = 20$; Figs. 7 and 8, showing polar view of MI with 20 bivalents; Fig. 9, polar view of early AI, showing 40 half-bivalents. ×1450

FIGS. 10–12. *Polytrichum ohioense* Ren. & Card., $n = 14$; Fig. 10, polar view of MI; Fig. 11, polar view of late MI, showing 14 bivalents; Fig. 12, polar view of AII, showing 56 chromosomes. ×1450

species, varieties, and forms have been described and the task of evaluating these multitudinous entities has scarcely begun. Numerous investigations have been made of its chromosomes and a part of the explanation of the enormous variability of *P. denticulatum* must lie in the fact that it is an extreme polyploid.

Vaarama (1950) and Yano (1957*a*, 1957*c*) reported the number $n = 10$ in Finland and Japan, respectively, but a population has been studied in the British Isles that showed, in addition to the 10 larger bivalents, a small precociously separating bivalent of the diminutive type (Vaarama 1956). In Finland, the var. *laetum*, which is also widely distributed in Eastern North America, has the number $n = 10+1$ (Vaarama 1950). A polyploid population, with the number $n = 20$, was discovered in the high mountains of the Southern Appalachians by Anderson and Bryan (1958) and a higher polyploid number, $n = 25$, was reported by Anderson and Crum (1959) from the Canadian Rockies.

It is not surprising to find the chromosome number $n = 20$ in a Quebec population to be the same as that in the Southern Appalachians, since the two regions have floristic resemblances. Plants in the two populations have been compared morphologically and they resemble one another closely. Both populations belong to the form of *P. denticulatum* that is moderately robust, occurs in bright green, somewhat glossy mats, complanate, with short-acuminate, almost acute leaves, denticulate at apex, with lower margins conspicuously recurved.

TABLE I

Chromosome numbers in some mosses from Quebec

| Name and source of material | Specimen number | Chromosome number | Figure |
|---|---|---|---|
| *Dicranum rugosum* (Hoffm.) Brid. | 14001 | $n = 12$ | 1, 2 |
| *Dicranum scoparium* Hedw. | 14002 | $n = 12$ | 3, 4, 5 |
| *Plagiothecium denticulatum* Hedw. | 14003 | $n = 20$ | 6 |
| *Plagiothecium roeseanum* (Hampe) B.S.G. | 14004 | $n = 20$ | 7, 8, 9 |
| *Polytrichum ohioense* Ren. & Card. | 14005 | $n = 14$ | 10, 11, 12 |

In the Quebec material, the meiotic chromosomes stain intensely in aceto-orcein, and, although crowded when viewed from the poles at metaphase, they are not sticky and do not adhere together. Meiotic chromosomes are thus easy to interpret and count. This seems to be true also of populations of this species studied by other investigators.

*Plagiothecium roeseanum* (Hampe) B.S.G., $n = 20$ (Figs. 7–9)

The chromosomes of this species are identical in number, behavior, and morphology with those of *P. denticulatum*, with which it is closely allied. Indeed, the distinctness of *P. roeseanum* from *P. denticulatum* is not well established taxonomically, and the former might well be treated as a subspecies or variety of the latter, a disposition suggested by Grout (1932). The close cytological resemblance supports this view.

In *P. roeseanum*, metaphase figures reveal 20 crowded but distinct bivalents, which stain vividly with aceto-orcein. There is not much variation in size of the bivalents, as all of them are extremely compact and condensed. A feature of the sporocytes of both *P. roeseanum* and *P. denticulatum* is the fact that meiosis proceeds almost simultaneously in all of the spore mother cells of the sporogonium. Also, in both species, unlike meiosis in many mosses, all of the bivalents disjoin very nearly at the same time. This is illustrated in Figs. 7 and 8, where all of the bivalents are shown in close association. Figure 9 shows a sporocyte in first anaphase in which all of the bivalents have disjoined so that 40 half-bivalents are clearly apparent. Such an appearance is typical of first anaphase figures that have been squashed. Apparently only slight pressures are required to dislodge anaphase chromosomes from the spindle and to destroy the typical anaphasic configuration. This situation can be misleading, since meiosis proceeds simultaneously in each capsule. We encountered preparations in which every sporocyte presented the appearance illustrated by Fig. 9, and, as can be seen, an erroneous chromosome count could result.

Additional chromosome studies of this species complex are awaited with considerable interest. Of especial significance would be a knowledge of the chromosome number of *P. laetum* in North America. The chromosome number of *P. ruthei* Limpr., one of the largest members of the complex, should prove enlightening in view of its *gigas*-like morphological characters. Cytological studies of many western populations are needed to throw light on the remarkable 25-chromosome population of *P. denticulatum* reported by Anderson and Crum (1959). One is tempted to predict the occurrence of both 10-chromosome and 15-chromosome ($n$) races in the Canadian Rockies.

*Polytrichum ohioense* Ren. & Card., $n = 14$ (Figs. 10–12)

This is the first report of the chromosome number of this North American species. The family Polytrichaceae, of which it is a member, is noted for uniformity of chromosome numbers. All of the members of the family that have been studied cytologically have the basic number $n = 7$, with a few species being tetraploid, $n = 14$. *Polytrichum ohioense* proves to be another tetraploid species. *Polytrichum gracile* Sm. and *P. yezoense* Hor. are other species with tetraploid numbers (Vaarama 1953; Holmen 1958; Yano 1957*d*).

The chromosomes of *P. ohioense* at first meiotic metaphase are quite condensed, with tightly associated bivalents. During anaphase I the chromatid configurations of the half-bivalents are conspicuous (Fig. 11). Meiosis is normal. Disjunction occurs almost simultaneously and no evidence of lagging or other irregularities were observed. Figure 12 shows a sporocyte during second anaphase in which 56 chromosomes can be counted.

It is significant perhaps that the tetraploid species of *Polytrichum* known at this time all belong to Section *Polytricha alpina* Hag., although the type species of the section, *P. alpina* itself, is a diploid species ($n = 7$), according to Yano (1957*d*).

## Acknowledgments

Grateful acknowledgment is made to the National Science Foundation for a grant of research funds to Duke University (NSF-G9610), which has made the present study possible. We are indebted to Dr. Howard Crum, National Museum of Canada, for aid in the collection of specimens.

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# STUDIES OF VIRUS DISEASES OF STRAWBERRIES IN BRITISH COLUMBIA

## III. TRANSMISSION OF STRAWBERRY VIRUSES BY APHIDS[1]

F. C. Mellor and A. R. Forbes


## Abstract

Eleven aphid species commonly found on rosaceous plants were tested as vectors of eight strawberry viruses. *Amphorophora rubi* (Kalt.), *Aphis rubifolii* (Thomas), *Aulacorthum solani* (Kalt.), *Macrosiphum rosae* (L.), *Myzus ascalonicus* Doncaster, *M. ornatus* Laing, *M. persicae* (Sulz.), *Pentatrichopus fragaefolii* (Cock.), *P. tetrarhodus* (Walk.), and *P. thomasi* Ris Lambers transmitted the vein banding virus from one or both of two sources. *P. fragaefolii*, *P. tetrarhodus*, and *P. thomasi* transmitted mild mottle, while only *P. fragaefolii* and *P. thomasi* transmitted curly-dwarf mottle. *M. rosae* transmitted vein banding and possibly mild yellow edge. *Aphis forbesi* Weed failed to transmit any of the viruses tested. None of the species tested transmitted witches' broom, crinkle, or strawberry latent-A virus.


## Introduction

Aphids have been implicated in the spread of strawberry viruses since 1927 when Plakidas (11) demonstrated the transmission of xanthosis (yellows) by the strawberry aphid, *Pentatrichopus fragaefolii* (Ckll.) (= *Myzus fragaefolii* Ckll., *Capitophorus fragaefolii* Ckll., *Capitophorus fragariae* Theo., *Pentatrichopus fragariae* Theob.). Subsequently this aphid was shown to transmit other strawberry viruses. Several other aphid species have also been shown to transmit some of these viruses but none so efficiently as *P. fragaefolii*.

This paper presents the results of experiments in which 11 species of aphids were tested as vectors of eight strawberry viruses. These aphids all occur on rosaceous plants and most are common on strawberry. Several are known vectors of strawberry viruses while others either have not been tested previously or have failed as vectors in earlier tests.

## Review of Literature

Following aphid transmission of xanthosis by Plakidas (11), separation of two component viruses of this disease by *P. fragaefolii* was demonstrated by Prentice & Harris (15) and Prentice (13). Persistence of the virus in the vector was the basis for the separation, one component being lost within a few hours, while the second persisted for several days. Similar separation of the components of xanthosis was reported by Mellor and Fitzpatrick (8).

Mottle is the non-persistent component of xanthosis. This term was first used by Thomas (17) to describe symptoms on *Fragaria vesca californica* which he had used to index apparently normal strawberry plants of the Marshall variety, and he also suggested that mottle was a component of

[1]Manuscript received February 10, 1960.
Contribution No. 13, Research Station, Canada Agriculture, c/o The University of British Columbia, Vancouver 8, B.C.

xanthosis. Subsequent work by other investigators confirmed his observations. Isolates of this component may differ greatly in the type and severity of symptoms they cause on the indicator, *F. vesca*, and they have received various names from different investigators: crinkle (19), mild crinkle (15), virus 1 (13), type 1 (1), and mottle (14, 17).

Frazier and Posnette (6) subdivided the mottle isolates into three forms: mottle, curly-dwarf mottle, and rusty-leaf mottle. All members of the mottle group are transmitted by the aphid after acquisition feeding periods of 1 hour and persist in the aphid for less than 6 hours. All are readily eliminated from infected plants by heat treatment. These similarities make impossible the separation of any component from the mixture of viruses or virus strains usually found in any mottle isolate except by chance separation during aphid transmission.

*P. fragaefolii* was the only vector reported for strawberry mottle, or indeed for any strawberry virus, until 1941 when Whitehead and Wood (19) reported transmission by the closely related species *P. tetrarhodus* Walk., a rose aphid. Since that time eight more vectors of this virus have been recorded. Frazier (3) reported transmission of non-persistent viruses, later identified as mottle, by three aphid species collected on strawberry: *Amphorophora rubi* (Kalt.), *Macrosiphum pelargonii* (Kalt.), and *Myzus ornatus* (Laing); and two species found only on cultivated rose: *Myzaphis rosarum* (Walk.), and *Myzus porosus* Sanderson. The following year Posnette (12) added two more species to Frazier's list of vectors: *Acyrthosiphon malvae* subsp. *rogersii* (Theob.) (= *M. pelargonii* ?), and *Myzus ascalonicus* Doncaster. Duffus' (2) report of transmission of mottle by *Aphis gossypii* Glover, confirmed by Frazier and Posnette (6), brought the number of vectors of mottle to 10.

Mild yellow edge is the persistent component of xanthosis. Prentice (13) found it could be acquired by the aphid in 24 hours and persisted in the vector for several days. This virus has been transmitted only by three closely related species of *Pentatrichopus*: *P. fragaefolii*, *P. thomasi* Ris Lambers, and *P. thomasi* subsp. *jacobi* Ris Lambers (6).

Vein banding is another virus with several vectors. This is a non-persistent virus of strawberry described by Frazier (5), who reported four vectors: *A. rubi*, *P. fragaefolii*, *M. pelargonii*, and *M. ornatus*. He found transmission by *P. fragaefolii* to be erratic, and of six colonies, each originating from aphids collected in a different locality, four were efficient vectors of vein banding while two did not transmit the virus. In a later publication Frazier and Posnette (6) identified the two non-transmitting colonies as *P. thomasi*, two of the vector colonies as *P. fragaefolii*, and the remaining two vector colonies as *P. t. jacobi*.

Vector relationships of this virus are similar to those of mottle (5), but unlike mottle, it is resistant to heat therapy (unpublished).

Vein banding is latent in commercial varieties of strawberry and causes only mild symptoms on *F. vesca*. When it is combined with strawberry latent-A virus, severe symptoms appear both on the commercial varieties and on the indicators (unpublished).

Witches' broom is a virus disease of strawberry first described in 1927 in Oregon by Zeller (20), who reported transmission by *P. fragaefolii* after an acquisition feeding period of 8 days. Since this early report the disease was apparently unnoticed until 1958 when it again appeared in the Pacific Northwest (9).

Crinkle was first described by Zeller and Vaughan (21) in 1932, and its transmission by *P. fragaefolii* was reported by Vaughan (18) the following year. Prentice and Woollcombe (16) demonstrated that the aphid could acquire the virus during a 24-hour acquisition feeding period, but that a latent period of 12 to 16 days in the vector must elapse before transmission could occur. Later attempts to transmit crinkle by the strawberry aphid were unsuccessful at East Malling (6) but the virus had been aphid-transmitted in California, although only by naturally infective aphids. Our own attempts to transmit this virus by aphids have all failed (unpublished). No vector other than *P. fragaefolii* has been reported.

Strawberry latent-A virus was reported by Frazier in 1953 (4). No vector has been found for this virus and it is apparently transmitted only by grafting. Latent-A is carried by the East Malling clone of *F. vesca* which has been widely distributed and used for graft-indexing of commercial strawberry varieties, so that this virus has been introduced into many planting stocks. It causes no symptoms either on commercial varieties or on the *Fragaria* species commonly used as indicators, but in combination with other viruses it intensifies the symptoms of the second virus.

## Materials

*Vectors*

The aphid species tested, their natural host plants, and the plants on which they were reared are given in Table I.

All but two of the species were reared from random collections. Since *Pentatrichopus fragaefolii* and *P. thomasi* differ only in chaetotaxy[2] they were each reared from a single viviparous female. Samples from all colonies were mounted for identification and reference.

*Virus Source Plants*

Eight virus isolates were used in these tests. Three were obtained from apparently normal strawberry plants of the British Sovereign variety. These were: mild mottle, curly-dwarf mottle, and vein banding (source 1). Four were obtained from strawberry plants with severe symptoms of virus degeneration. These were: mild yellow edge and crinkle from plants of the Marshall variety; and witches' broom and a second isolate of vein banding (source 2)

[2]Two very similar species of *Pentatrichopus* living on *Fragaria* were discovered by Thomas and Jacob in North America. The main difference between the two species is the presence of two rows of marginal hairs on the anterior abdominal tergites in one species, and four rows of such hairs in the other. Both species were identified as *fragaefolii* Cock. Comparison of the chaetotaxy of the first species with that of the European strawberry aphid led Hille Ris Lambers (7) to believe the name *Pentatrichopus fragaefolii* (Cock.) was justified for the European species, and he suggested the new name *Pentatrichopus thomasi* for the second species with the double pair of marginal hairs.

TABLE I

Aphid species tested as vectors of strawberry viruses

| Aphid species | Natural host plants | Laboratory host plant |
|---|---|---|
| *Amphorophora rubi* (Kalt.) | Raspberry | Rapsberry infected with unidentified virus |
| *Aphis forbesi* Weed | Strawberry | Strawberry, var. British Sovereign (virus-free) |
| *Aphis rubifolii* (Thomas) | Raspberry | Raspberry (virus-free) |
| *Aulacorthum solani* (Kalt.) | Include strawberry | *Nicandra physaloides* (virus-free) |
| *Macrosiphum rosae* (L.) | Rose | Garden rose (virus content unknown) |
| *Myzus ascalonicus* Doncaster | Include strawberry | Onion (virus-free) |
| *Myzus ornatus* Laing | Include strawberry | Pansy seedlings (virus-free) |
| *Myzus persicae* (Sulz.) | Many, but do not include strawberry | Rutabaga (virus-free) |
| *Pentatrichopus fragaefolii* (Cock.) | Strawberry | *Fragaria vesca*, var. Alpine (virus-free) |
| *Pentatrichopus tetrarhodus* (Walk.) | Rose | *Rosa rugosa* (virus content unknown) |
| *Pentatrichopus thomasi* H.R.L. | Strawberry | *Fragaria vesca*, var. Alpine (virus-free) |

from a plant of the British Sovereign variety. The eighth virus, strawberry latent-A, was obtained from the East Malling clone of *Fragaria vesca*.

The virus source plants used for these tests, except those of crinkle and latent-A, were all plants of a latent-free clone of *F. vesca*. A single plant was used as the source of each virus throughout the tests so that, if a mixture of viruses were present, each aphid species tested would have access to the same mixture.

The two mottle isolates were both originally transmitted by *P. fragaefolii* from an apparently normal British Sovereign to a plant of the East Malling clone of *F. vesca*. The presence of a mixture of viruses or virus strains in this plant was shown by subsequent aphid transmissions which caused a range of symptoms on the indicators which, on latent-free *F. vesca*, varied from a very mild mottle with no leaf distortion and little or no loss of vigor, to extreme degeneration; and on latent-carrying *F. vesca*, varied from mild mottle to extreme degeneration, and often death.

The mild mottle isolate used in these tests was separated by aphid transmission. In subtransfers of the mild mottle, symptoms were consistently mild. On latent-A-free *F. vesca* the mottle was faint, and even on the latent-A-carrying East Malling *F. vesca* the mottle was mild, with virtually none of the "scar-tissue" spots usually associated with mottle plus latent-A (6).

The curly-dwarf isolate used in these tests was the result of two further subtransfers by *P. fragaefolii* from the original isolate. In contrast to the mild mottle, transmissions from curly-dwarf cause a range of symptoms on the indicator, and a mixture of viruses or virus strains in this isolate is probable.

Mild yellow edge virus was transmitted by *P. fragaefolii* from a Marshall plant with xanthosis to East Malling *F. vesca*. The separation of this virus from xanthosis and description of its symptoms are given in an earlier paper (8).

Vein banding source 1 was originally transmitted from an apparently normal British Sovereign plant to East Malling *F. vesca* by *P. fragaefolii*. Subtransfers from this source caused symptoms which frequently varied in severity, although not to the extent shown by symptoms of mottle. This variation was particularly noticeable on latent-carrying *F. vesca*.

Vein banding source 2 was obtained from the same plant that served as the source of witches' broom. In earlier tests aphid transmission of vein banding from this source caused symptoms milder than those usually caused by vein banding from source 1. For this reason, and because differences in aphid transmission from the two sources appeared in the present series of tests, the two sources of vein banding are considered separately.

Witches' broom virus was obtained from an infected British Sovereign plant which also carried mottle and vein banding. Heat treatment eliminated the mottle but no way was found to separate witches' broom from the vein banding and, at the time these tests were started, no source of witches' broom without vein banding was available.

Crinkle virus was transmitted by grafting from an infected Marshall to East Malling *F. vesca*. Since we have never succeeded in transmitting this virus by means of aphids, it could not subsequently be separated from latent-A by aphid transmission to a latent-free indicator, and the source plant of crinkle for these tests was an East Malling *F. vesca*.

Strawberry latent-A virus was obtained from the East Malling clone of *F. vesca*.

*Indicator Plants*

The indicators for all but two of the viruses were *F. vesca* of the East Malling clone which, because it carries strawberry latent-A virus, reacts to infection by some viruses with symptoms more definite than those on indicators without latent-A. For latent-A itself the indicators were plants of a clone developed from a latent-A-free *F. vesca* infected with vein banding. For mild yellow edge the indicators were seedlings of the runnerless Alpine *F. vesca*.

## Methods

Six indicator plants were inoculated from each of the virus sources by each of the aphid species. In most cases the indicators were inoculated twice. Leaves were detached from each source plant and supported in vials of water, and 125 aphids of the species under test were placed on the leaves. After an acquisition feed of 1 day six aphids were moved to each indicator, confined in leaf clip cages (10), and allowed to feed for 4 days. These aphids were then killed and, if the number of surviving aphids permitted, a second group of six aphids, having had an acquisition feed of 5 days, were moved to each of the same indicators and allowed to feed for 10 days.

There were enough survivors after the 1-day acquisition feed to inoculate all the plants, but the second inoculation was not always possible with those species which do not normally colonize on strawberry. There were few survivors of *A. rubi*, *M. rosae*, and *M. persicae* after the 5-day acquisition feed and only

a few plants received the second inoculation by these three species. *A. rubifolii* and *P. tetrarhodus* did not survive on the virus sources for 5 days and there were no second inoculations.

To detect toxicity from aphid feeding, or virus transmission from host plants on which the aphids were reared, a comparable group of aphids of each species was allowed to feed on detached leaves of virus-free Alpine *F. vesca* before being moved to six indicators.

To confirm unusual or negative results, some of these tests were repeated.

## Results and Discussion

All the aphid species tested except *A. forbesi* transmitted one or more strawberry viruses. Six new vectors of vein banding and one of mild yellow edge were discovered. Several previous records of virus transmissions were not confirmed in these tests. However, the number of test plants was small and the negative results are not necessarily significant. New records and other results of special interest are discussed below. Detailed results are given in Table II.

Mottle was transmitted only by *Pentatrichopus* spp.; the mild mottle isolate by *P. fragaefolii*, *P. thomasi*, and *P. tetrarhodus*; the curly-dwarf isolate by *P. fragaefolii* and *P. thomasi*. The symptoms of mild mottle were similar regardless of the vector. The symptoms of curly-dwarf mottle, on the other hand, were milder when the virus was transmitted by *P. thomasi* than when it was transmitted by *P. fragaefolii*, and lacked the initial necrosis that usually occurs when curly-dwarf mottle is transmitted to East Malling *F. vesca* either by *P. fragaefolii* or by grafting. The curly-dwarf isolate used in these tests is probably a mixture of viruses or virus strains and it may be that *P. thomasi* is unable to transmit part of the mixture.

Three aphid species previously reported as vectors of mottle (3, 12) failed to transmit either of the two isolates. These are: *A. rubi*, *M. ascalonicus*, and *M. ornatus*.

Mild yellow edge was transmitted in only one instance, by *M. rosae*. Since the host plant from which this species had been obtained was a garden rose whose freedom from virus was not known, and since *M. rosae* was not previously reported to be a vector of mild yellow edge, the single transmission was questioned even though symptoms were characteristic and there were no transmissions to the check plants. However, a repetition of this test brought about no further transmissions and neither did a test in which 12 *M. rosae* were transferred directly from the same rose plant to each of six indicators.

The failure of *Pentatrichopus* spp. to transmit mild yellow edge is difficult to explain. *P. fragaefolii* and *P. thomasi* have both been reported as vectors of this virus (13, 6), and during early work on strawberry viruses at Vancouver, it was aphid-transmitted without difficulty. For example, in an experiment in 1951 in which 163 plants were inoculated by three aphids each, 112 became infected. During recent years, however, we have had little success with aphid transmission. In 1955 the ability of six aphid clones to transmit mild yellow edge was compared. Each clone had been reared from an apterous viviparous

TABLE II

Transmission* of strawberry viruses by 11 species of aphids

| | Mottle | | Mild yellow edge | Vein banding | | Witches' broom | Crinkle | Latent-A |
|---|---|---|---|---|---|---|---|---|
| | Mild mottle | Curly-dwarf | | Source 1 | Source 2 | | | |
| *Amphorophora rubi* (Kalt.) | 0/6‡ | 0/6‡ | 0/6 | 0/6 | 2/6 | 0/6 | 0/6 | 0/6 |
| *Aphis forbesi* Weed | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 |
| | | | | 0/6 | 0/3 | 0/3 | | |
| *Aphis rubifolii* (Thomas) | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 |
| | | | | 2/6 | 0/6 | 0/6 | | |
| *Aulacorthum solani* (Kalt.) | 0/6 | 0/6 | 0/6 | 1/6† | 2/6† | 0/6 | 0/6 | 0/6 |
| *Macrosiphum rosae* (L.) | 0/6 | 0/6 | 1/6† | 1/6† | 2/6† | 0/6 | 0/6 | 0/6 |
| | | | 0/6 | | | | | |
| *Myzus ascalonicus* Doncaster | 0/6‡ | 0/6‡ | 0/6 | 3/6† | 1/6† | 0/6 | 0/6 | 0/6 |
| *Myzus ornatus* Laing | 0/6‡ | 0/6‡ | 0/6 | 5/6 | 0/6 | 0/6 | 0/6 | 0/6 |
| | | | | 4/6 | 0/6 | 0/6 | | |
| *Myzus persicae* (Sulz.) | 0/6 | 0/6 | 0/6 | 2/6† | 0/6 | 0/6 | 0/6 | 0/6 |
| *Pentatrichopus fragaefolii* (Cock.) | 5/6 | 6/6 | 0/6‡ | 6/6 | 6/6 | 0/6‡ | 0/6‡ | 0/6 |
| *Pentatrichopus tetrarhodus* (Walk.) | 1/6 | 0/6 | 0/6 | 2/6† | 5/6† | 0/6 | 0/6 | 0/6 |
| *Pentatrichopus thomasi* H.R.L. | 6/6 | 6/6 | 0/6‡ | 5/6† | 0/6 | 0/6 | 0/6 | 0/6 |
| | | | | 5/6 | 0/6 | 0/6 | | |

*The numerator is the number of plants showing symptoms; the denominator is the number of plants inoculated. Where tests were repeated, results of the second test are given below those of the first.
†New record.
‡Previously reported vectors.

female with capitate hairs, collected from strawberry, and on this basis all were believed to be *P. fragaefolii*. In this experiment the acquisition feeding period was 6 days, the transfer feeding period 4 days, and the number of aphids per plant was 10. The number of transmissions by each aphid clone was as follows: 0/8, 2/14, 2/4, 1/12, 5/16, and 6/12, where the numerator indicates the number of transmissions, and the denominator the number of plants inoculated.

The clone which did not transmit (clone A) is of particular interest because it continued viviparous reproduction throughout the year. All other clones we have used have been lost when sexual forms appeared in October. New clones were sometimes started from adults collected in the field, but usually they originated from eggs collected from the field in early spring and hatched in the laboratory. Clone A was confirmed as *P. fragaefolii* by Hille Ris Lambers in 1955. In view of the continuing viviparous reproduction of clone A, the oviparous reproduction of the other clones casts doubt on their identity.[3]

*P. fragaefolii* clone A was a consistently poor vector of mild yellow edge. In only one test did it transmit satisfactorily, when four out of five inoculated plants became infected. In other tests a total of 45 plants were inoculated with no transmissions. During the present series of tests, clone A was the only colony of *P. fragaefolii* available. As in previous years, it proved to be a good vector of mottle, curly-dwarf mottle, and vein banding, but again it failed to transmit mild yellow edge.

The erratic transmission of this virus is unexplained, but it is of interest that transmission of mild yellow edge by *P. fragaefolii* at East Malling, England, and by *P. t. jacobi* at Berkeley, California, has been similarly erratic (6).

Vein banding was transmitted by all the aphid species tested except *A. forbesi*, although not always from both sources. The difference in number of transmissions of this virus from the two sources was particularly marked for *P. thomasi* and *M. ornatus*. From source 1 *P. thomasi* transmitted the virus to 10 out of 12 test plants; *M. ornatus* to 9 out of 12. From source 2 neither species transmitted the virus to any of 12 plants. Furthermore, in most cases, the virus from source 1 caused more severe symptoms than the virus from source 2, confirming previous observations on the relative virulence of vein banding from the two sources.

Vein banding has already been shown to have a wide range of vectors (5, 6) which is extended by these results. Aphids reported here for the first time as vectors of vein banding are: *A. solani*, *M. rosae*, *M. ascalonicus*, *M. persicae*, *P. tetrarhodus*, and *P. thomasi*.

[3]In summarizing the biology of *P. fragaefolii*, Hille Ris Lambers (7) observed that in the open the species does not normally form sexuales. In the laboratory many sexuales are frequently formed during the winter but these usually produce non-fertile eggs. Hibernation as eggs has not been observed in Europe. In North America sexual reproduction is said to occur normally. However, because it is impossible to determine whether the American papers on *fragaefolii* refer to true *P. fragaefolii* or to *P. thomasi*, new investigations are necessary before data about hibernation of *P. fragaefolii* as eggs can be accepted by Hille Ris Lambers.

Crinkle was not transmitted by any of the aphids in the present tests, although transmission of this virus by *P. fragaefolii* has been reported (6, 16, 18).

Although six aphid species transmitted vein banding from the witches' broom source, none transmitted the witches' broom virus itself. Transmission of the witches' broom virus by *P. fragaefolii* has been reported (20).

Latent-A was not transmitted by any of the aphids tested; nor have other workers succeeded in transmitting this virus by aphids.

There was no evidence of toxicity from aphid feeding, nor were there any transmissions from the laboratory host plants.

## Conclusions

The most efficient vectors of strawberry viruses in these tests were *P. fragaefolii* and *P. thomasi*. The viruses most readily transmitted by aphids were vein banding and mottle.

Vein banding was transmitted by 10 of the 11 species of aphids tested. The frequency with which this virus is transmitted has special significance in the field where commercial varieties carrying vein banding may be grown near other varieties carrying latent-A. Either of these viruses alone is symptomless, but when vein banding is transmitted to plants carrying latent-A, severe symptoms quickly appear and degeneration follows.

The ability of the vectors *M. ornatus* and *P. thomasi* to transmit vein banding from one source but not from another indicates an unresolved difference in the vein banding virus from the two sources.

The efficiency of *P. fragaefolii* as a vector of vein banding from both sources, while *P. thomasi* was unable to transmit the virus from the second source, indicates there may be biological as well as morphological differences between the two species. Frazier and Posnette (6) suggest that inability to transmit vein banding virus may perhaps prove to be a characteristic of *P. thomasi*.

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# COLD HARDENING AND COLD HARDINESS OF YOUNG WINTER RYE SEEDLINGS AS AFFECTED BY STAGE OF DEVELOPMENT AND TEMPERATURE[1]

J. E. ANDREWS


## Abstract

Young winter rye seedlings, grown and hardened at 1° or 1.5° C in the dark, developed a high level of cold hardiness at two stages prior to emergence of the first leaf. The first maximum occurred when coleoptiles were less than about 1 mm in length and was followed by a decrease in hardiness. A second and higher maximum occurred when coleoptiles were about 15–30 mm in length (5 weeks at 1.5° C; 7 weeks at 1° C) and it was followed by a rapid decrease in hardiness beginning at about the time the leaf broke through the coleoptile. Genetic differences corresponding with those obtained in the field were established by hardening seedlings for 7 weeks at 1° C and exposure to −15° C for 16 hours or by hardening for 5 weeks at 1.5° C and exposure to −14° C for 16 hours. The use of a lower (−4° C) hardening temperature resulted in a large increase in cold hardiness at the younger stages of development but little or no increase where seedlings had already reached a maximum of hardiness from exposure to 1.5° C for 5 weeks. Satisfactory genetic differences were not determined by exposure to −14° C for 16 hours after hardening at −4° C. In general the response to hardening of young winter rye seedlings was similar to that found with winter wheat.


## Introduction

It is widely recognized that an increased knowledge of cold hardening, cold hardiness, and reliable methods of identifying genetic differences in cold hardiness is of considerable fundamental importance in the development of winter hardy cereal crops. Extensive reviews of the literature on the nature and practical evaluation of winter hardiness in plants have been published by Dexter (4) and Levitt (6).

The literature on cold hardiness of very young seedlings was reviewed by Andrews (1, 2) and Dantuma and Andrews (3). Results of studies with seedlings are of considerable interest to plant breeders because testing for genotypic differences at that stage saves time and space as compared with older plants. Andrews (1) showed that genetic differences could be determined by appropriate hardening and freezing of winter wheat during sprouting. The relative differences between varieties found at that stage were similar to those obtained from winter hardiness tests in the field. Andrews (2) found that very young winter wheat seedlings would harden in the dark and that the level of hardiness obtained was greatly influenced by hardening temperature, stage of growth, and duration of hardening. Maximum hardiness was obtained after about 6 weeks of hardening at 1.5° C, when coleoptiles were 15–25 mm in length. There also was an indication in some experiments that very young seedlings (coleoptiles—1 mm) develop a high level of hardiness. Segeta (7) found high resistance to low temperatures in sprouting winter wheat and rye at a stage just before the coleoptiles and primary roots ruptured

[1]Manuscript received January 11, 1960.
Contribution from the Cereal Breeding Section, Canada Agriculture Research Station, Lethbridge, Alberta.

the pericarp. He was able to determine varietal differences at that stage. Dantuma and Andrews (3) found that winter wheat had a high level of cold hardiness after 1 week of growth and hardening at 1.5° C. This was followed by a rapid decrease to a minimum at 2–3 weeks, then an increase to a second maximum at 6–7 weeks, and then another rapid decrease in cold hardiness. Additional hardening at −4° C resulted in a large increase in cold hardiness at the younger stages of germination but only a slight increase at stages reached after 5 weeks at 1.5° C.

The experiments reported herein were undertaken to investigate the response of winter rye varieties to various hardening treatments during the early stages of germination and to determine the feasibility of identifying genetic differences at this stage of development.

## Materials and Methods

The varieties used in these experiments represented a wide range of winter hardiness. Ranked in order of field winter hardiness, the rye varieties were Antelope, Sangaste, Petkus, Dominant, and Abruzzi. The difference between Petkus and Dominant is small. Three varieties of winter wheat were included in some experiments for comparison. These were Kharkov 22 M.C. and Minturki, which have a high level of field winter hardiness, and Elgin, which has a much lower level of hardiness. All seed was produced in the same year, hand-picked for freedom from cracks and chips, and treated with Ceresan.

Two types of plastic containers with hinged lids, one $3\frac{1}{2}\times7$ in. with 10 compartments, as illustrated in Fig. 1, and the other $4\frac{1}{2}\times8$ in. with 18 compartments, were used in the experiments. Ten cubic centimeters of dry vermiculite (finish aggregate grade) were placed in each compartment as a substrate for growth and 8 ml of distilled water were added to each compartment. About two hours later the seeds were placed on the surface of the moist substrate. In all experiments there were 20 seeds of a variety per box compartment and either 9 or 10 replicates. During the hardening and freezing treatments the boxes were kept in individual polyethylene bags to prevent evaporation around the lids.

Except where otherwise mentioned, germination was allowed to start at room temperature, about 20° C, for 16 hours and then the boxes were moved to a dark controlled-temperature chamber for hardening treatments. The hardening temperature and duration varied with experiments. The freezing treatment for all experiments was 16 hours in a freezing chamber controlled at either −15° or $-14°\pm0.5°$ C. After removal from the freezing chamber seedlings were allowed to thaw for about five hours at about 20° C, then were covered with a sand–vermiculite mixture and moved to a growth chamber controlled at 15° C and with 18 hours of light per day.

Survival counts were made about two weeks after exposure to freezing. All percentage data were transformed by means of the angular transformation (angle = arc sign/percentage) before the analysis of variance was applied. The multiple range test described by Duncan (5) was used to test significant

differences among varieties. In all tables where this test was applied transformed means of percentage survival are listed and any two means *not underscored* by the same line are *significantly different* at the 5% level and any two means *underscored* by the same line are *not significantly different.*

## Results

In the first experiment five rye varieties were grown and hardened at 1° ± 0.5° C for periods of 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 weeks. The stage of development reached at the end of each of these hardening treatments is illustrated in Fig. 1. Seedlings from all hardening treatments were exposed simultaneously to −15° C for 16 hours. The survival in all treatments is shown graphically in Fig. 2. Survival for all varieties was relatively high after 1 week of hardening but differences between varieties were small at that stage. For all varieties except Antelope there was a decrease in survival at the second and third week of growth and hardening. This was followed by a gradual increase in hardiness of all varieties except Abruzzi up to the sixth or seventh week of growth and hardening and, then, a second decrease. Abruzzi was apparently unable to develop sufficient cold hardiness to survive a freezing temperature of −15° C. Differences between varieties and between treatments were significant ($P<.01$). On the average, varieties reached their maximum hardiness between the sixth and seventh week. Data for these two treatments were combined and the survival means after angular transformation for the five varieties are given below.

| Antelope | Sangaste | Petkus | Dominant | Abruzzi |
|---|---|---|---|---|
| 51.8 | 28.3 | 20.3 | 14.0 | 0 |

Duncan's multiple range test (5) indicated significant differences among all varieties, and the varieties were ranked in the same order of relative hardiness as in field tests.

In a second experiment three wheat and three rye varieties were included to allow comparison of the response to hardening of the rye varieties with that of wheat. Considerable information was obtained for wheat varieties alone in previous studies (2, 3). The following three series of hardening treatments were used:

(A) hardening at 1.5° C for periods of 1, 2, 3, 4, 5, 6, 7, and 8 weeks;

(B) hardening at 1.5° C for periods of 1, 2, 3, 4, and 5 weeks followed by 1 week of additional hardening at −4° C; and

(C) hardening at 1.5° C for periods of 1, 2, 3, 4, and 5 weeks followed by 3 weeks of additional hardening at −4° C.

All treatments were exposed simultaneously to −14° C for 16 hours. It should be noted that both the initial hardening and freezing temperatures in this experiment were slightly higher than those of the first experiment.

The survival percentages are shown graphically in Fig. 3. With a few minor exceptions, which may be explained by the slightly higher hardening and freezing temperatures, the response to hardening at 1.5° C was similar to that obtained in the first experiment. The results obtained for rye and wheat

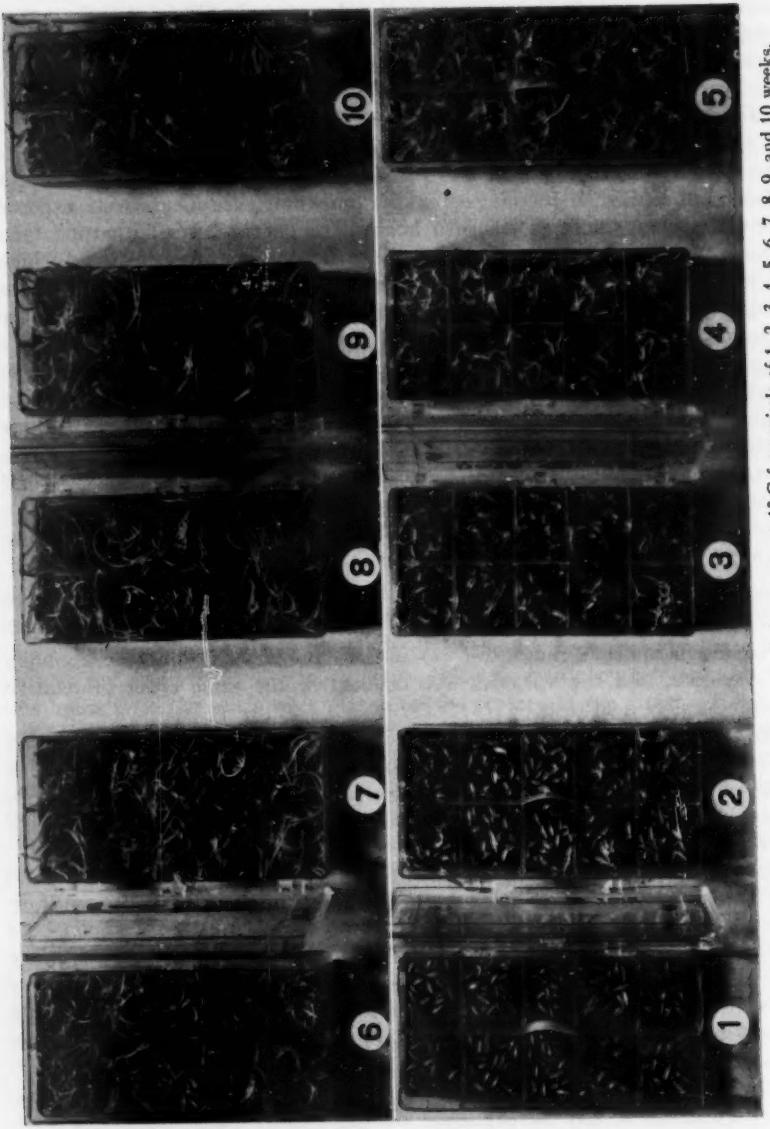


FIG. 1. Relative stages of development of rye varieties grown at 1° C for periods of 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 weeks.

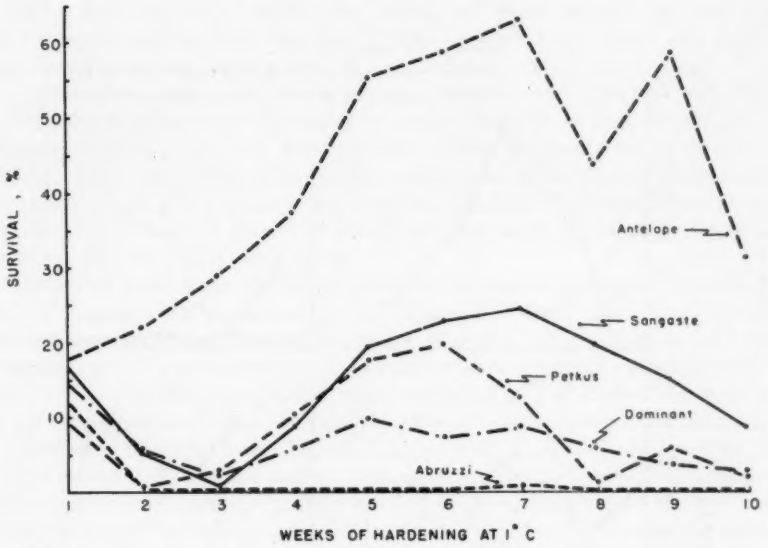


FIG. 2. Percentage survival of five winter rye varieties hardened at 1° C for periods of 1–10 weeks and exposed to −15° C for 16 hours.

varieties were generally similar. There was a rapid increase in the percentage survival of the hardy varieties during the first 5 or 6 weeks of hardening at 1.5° C and then a rapid decrease. Elgin, which is relatively low in winter hardiness, did not exceed 5% survival at any of the hardening periods at 1.5° C. Only Petkus had a higher survival at 1 than at 2 weeks of growth and hardening at 1.5° C, and all varieties reached maximum hardiness with a slightly shorter duration of hardening (5–6 weeks) than in the first experiment (6–7 weeks). This apparently resulted from the fact that growth was more rapid at 1.5° C than at 1° C. Consequently the first phase at which seedlings may exhibit a high level of hardiness was already passed when they were exposed to −14° C, and the second stage of maximum hardiness was reached sooner. Similar results were reported previously for wheat (2). The slightly higher freezing temperature (−14° C) apparently also resulted in slightly higher average survival of all varieties and smaller differences between varieties. The transformed survival means for varieties at 5 weeks of hardening, where maxima were reached for most varieties, and the combined means at 4, 5, and 6 weeks of hardening are given below, with differences indicated by the multiple range test.

| Duration at 1.5° C | Elgin | Petkus | Sangaste | Kharkov | Minturki | Antelope |
|---|---|---|---|---|---|---|
| 5 weeks | 6.6 | 50.1 | 51.7 | 52.4 | 63.2 | 68.4 |
| Means of 4, 5, and 6 weeks | 4.7 | 40.5 | 45.3 | 52.6 | 60.6 | 64.3 |

FIG. 1. Relative stages of development of rye varieties grown at 1° C for periods of 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 weeks.

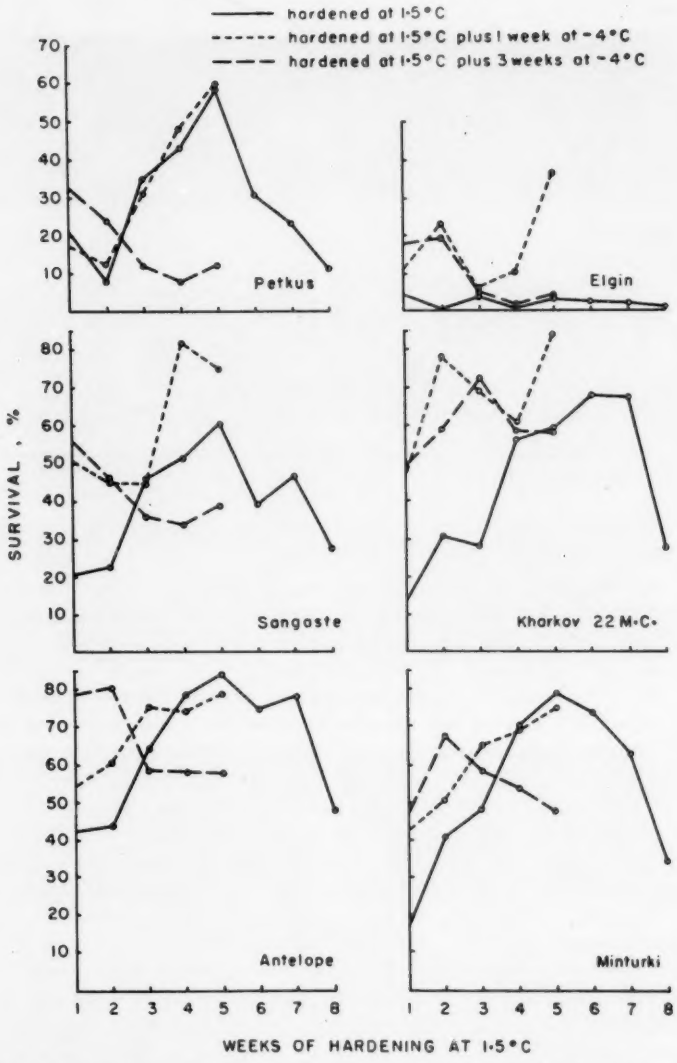


FIG. 3. Percentage survival of winter rye and wheat varieties following various hardening treatments and exposure to −14° C for 16 hours.

There were significant differences among all three wheat varieties. Of the rye varieties, Antelope had the highest survival, and Petkus was lower than Sangaste but not significantly so. Few data are available for a comparison of survival of the wheat and rye varieties in the field. At Lethbridge, Alberta, in 1956 the survival of the rye varieties and of Kharkov 22 M.C. wheat in an adjacent but different test was: Antelope 99%, Sangaste 76%, Kharkov 22 M.C. 71%, and Petkus 54%. In the young seedling test, Antelope had the highest survival and the surviving Antelope seedlings in general were more vigorous than those of wheat. A difference between Sangaste and Kharkov 22 M.C. was not clearly established.

There was some variation in response of varieties to additional hardening at −4° C after first hardening at 1.5° C, but in general their reaction was similar. One additional week of hardening at −4° C after prehardening 1 or 2 weeks at 1.5° C produced a large increase in survival of all varieties except Petkus. At the older stages additional hardening at −4° C resulted in little or no increase in survival of the hardier varieties but a considerable increase in survival of Elgin. Exposure to −4° C for 3 weeks as an additional hardening treatment resulted in some further increase in survival at the younger stages of development of Antelope and Petkus and no change in survival of the other varieties and, at the older stages, resulted in decreased survival of all varieties. It appears that exposure to −4° C either caused some damage to the older seedlings or advanced their physiological development to a stage at which they no longer had high resistance to the freezing temperature.

Although additional hardening at −4° C resulted in a considerable increase in survival of all varieties exposed to −14° C for 16 hours, varieties were not as well differentiated after this treatment as with hardening only at 1.5° C. The transformed survival means for the varieties with 1 and 3 weeks of additional hardening at −4° C after prehardening 1 week at 1.5° C are given below, with differences indicated by the multiple range test.

| Hardening and treatment | Elgin | Petkus | Minturki | Kharkov | Sangaste | Antelope |
|---|---|---|---|---|---|---|
| 1 week at 1.5° C plus 1 week at −4° C | 12.4 | 22.0 | 39.9 | 43.1 | 45.4 | 47.4 |
| 1 week at 1.5° C plus 3 weeks at −4° C | 21.6 | 34.2 | 43.0 | 44.2 | 48.2 | 63.7 |

In the first experiment the rye varieties had a relatively high level of cold hardiness after 1 week at 1° C and this was followed by a decrease in hardiness of all except Antelope. In the second experiment, all except one variety had apparently passed this stage of high hardiness after 1 week at 1.5° C. Therefore a third experiment was carried out to investigate further the ability of rye and wheat varieties to develop cold hardiness in the very young stages of germination. It included three rye and three wheat varieties. All were allowed to start germination for 8 hours at about 20° C and then were subjected to the following two series of hardening treatments:

(A) hardening at 1.5° C for periods of 0, 3, 6, 9, and 12 days and

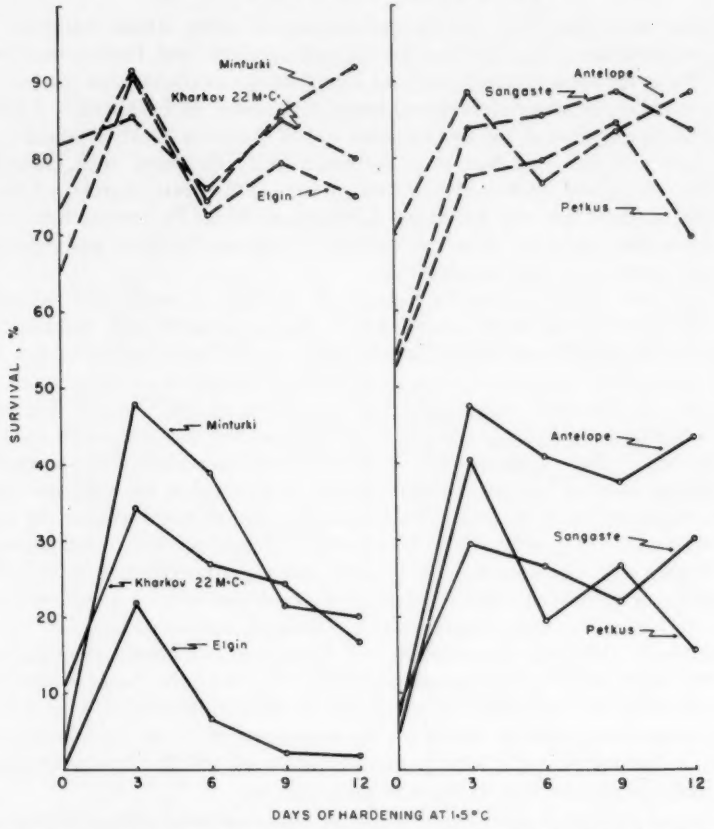


FIG. 4. Percentage survival of winter rye and wheat varieties following various hardening treatments and exposure to −14° C for 16 hours.
— — — With additional hardening for 2 weeks at −4° C.
——— Without additional hardening.

(B) hardening at 1.5° C for periods of 0, 3, 6, 9, and 12 days followed by 2 weeks at −4° C.

The checks (0 days at 1.5° C) were germinated for 24 hours at 20° C. Seedlings from all treatments were exposed simultaneously to −14° C for 16 hours. The survival percentages for individual varieties are given graphically in Fig. 4. The relative survival and condition of survival from certain treatments 2 weeks after freezing is illustrated in Fig. 5. The survival means (percentage transformed) for each hardening treatment are given below.

| Additional hardening treatment | Days of hardening at 1.5° C | | | | | S.E. | L.S.D. |
|---|---|---|---|---|---|---|---|
| | 0 | 3 | 6 | 9 | 12 | | |
| None | 8.8 | 35.5 | 27.4 | 24.1 | 23.2 | 3.4 | 9.5 |
| 2 weeks at −4° C | 56.8 | 71.2 | 64.4 | 70.6 | 67.5 | 4.1 | 10.1 |

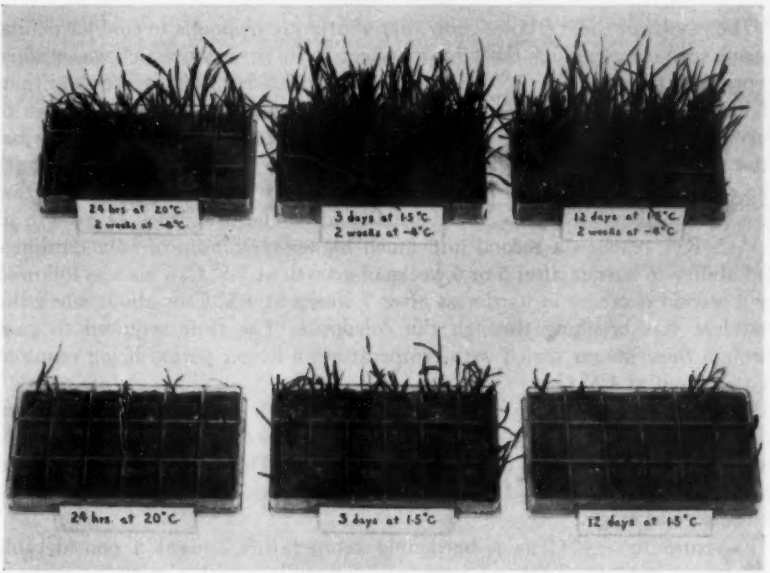


FIG. 5. Relative survival and condition of survivors from controls and certain hardening treatments 2 weeks after exposure to −14° C for 16 hours.

There was a higher survival of seedlings that received the hardening treatments than of the checks, grown for 24 hours at 20° C, indicating that rye and wheat will respond to hardening at these very young stages of development and that the hardiness exhibited is not directly carried over from the dormant seed. The mean survival of seedlings grown only at 1.5° C was highest at 3 days of growth and was reduced significantly by the ninth day. The reduction generally was greater on the wheat than on the rye varieties (Fig. 4). There were significant differences ($P<.05$) among all three wheat varieties at 3 and 6 days of hardening at 1.5° C. At 9 and 12 days of hardening there was no difference between Kharkov and Minturki but Elgin was significantly lower in survival (Fig. 4). Among the rye varieties Antelope had the highest survival for all hardening periods at 1.5° C. Sangaste and Petkus were significantly different only with 12 days of hardening at 1.5° C (Fig. 4).

Exposure to −4° C as a hardening temperature with or without prehardening at 1.5° C resulted in a high level of survival but varietal differences were not distinct (Fig. 4). A lower freezing temperature for seedlings hardened at −4° C might have given better varietal differentiation. Survival of seedlings hardened at −4° C was higher when they had first been prehardened for 3 days at 1.5° C than when they had received no prehardening treatment.

## Discussion

The results of these studies show that winter rye responds to cold hardening during sprouting in much the same manner as winter wheat, which was studied in previous experiments (2, 3). The rye varieties, under appropriate conditions of hardening, had a high level of cold hardiness at at least two phases of development prior to the emergence of the first leaf. The first occurred just after germination began, when coleoptiles were about 1 mm or less in length and roots less than about 5–10 mm in length. This was followed by a reduction in cold hardiness, which occurred usually after the first week of growth at 1.5° C. Rye reached a second and much higher maximum of cold hardiness and ability to harden after 5 or 6 weeks of growth at 1.5° C. This was followed by a second decrease in hardiness after 7 weeks at 1.5° C or about when the first leaf was breaking through the coleoptile. The time required to pass through these stages varied with temperature, a longer period being required at 1° C than at 1.5° C.

Genetic differences may be determined at both stages where maximum hardiness occurs, but for routine testing the second stage appears more satisfactory even though it requires more time. The first stage was passed through rather rapidly and differences between varieties were not as great as at the second stage.

Exposure to −4° C as a hardening temperature caused a considerable increase in cold hardiness of very young seedlings either with or without prehardening at 1.5° C. Little or no increase in cold hardiness occurred in seedlings that had already reached a maximum of cold hardiness from growth and hardening for 5 weeks at 1.5° C. Satisfactory genetic differences were not obtained when seedlings were hardened at −4° C and exposed to −14° C as a freezing temperature. However, it seems possible that the use of an appropriate low freezing temperature might have revealed differences. These and previous studies (2, 3) indicate that satisfactory separation of genotypes depends upon the use of an appropriate freezing temperature for the particular hardening treatments used and also for the general level of hardiness of the varieties involved.

Winter rye varieties such as Antelope are usually considered to have much more winter hardiness than winter wheat. Little comparative winter survival data for wheat and rye varieties are available but winter rye is produced successfully in areas of Western Canada where winter wheat will not survive. In the seedling studies reported herein, Antelope had the highest survival but the difference between it and the hardiest wheat varieties was not great. It is possible that the cold hardiness of rye is not manifest to the same extent in young seedlings as in older plants, or that additional factors are involved in the field, or that the hardening and freezing treatments used in the seedling studies might not have been appropriate to reveal true differences between the two species. However, differences between the rye varieties in the young seedling stages were similar to those obtained in field tests. Therefore, testing seedlings for genetic differences in cold hardiness should provide a reliable means of eliminating genotypes for low winter hardiness in rye-breeding programs.

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# THE LINKAGE MAP OF CHROMOSOME 2 OF THE TOMATO[1]

L. BUTLER


## Abstract

The data accumulated over the past 30 years from the classification of some half a million plants are presented. It is shown that the mapping of chromosome 2, the former linkage group 1, presents many difficulties because of the lethal nature of *Wo*, and the reduced viability of *ro*, *dv*, and *m*. The problem of classification of *o*, *bk*, and $ms_{10}$ also presents difficulties. The examination of the monogenic ratios shows many of them to be disturbed because of lethality, viability, and misclassification, but these causes do not account for all the discrepancies. In the case of *Wo* and *aw* there are large excesses of the dominant class; this evidence along with data from anomalous recombination values indicates a chromosomal abnormality. It is suggested that this abnormality is a duplication which in some stocks gives two *Wo* loci about 25% apart, and two *aw* less than 40% apart, and also involves the loci of *Me* and *Cu*.

The recombination values indicate that the map is *dv* 3 *m* 2 *d* 4 *p* 10 *aw* (*ps o ro*) 13 *Me* (*Cu bk*) 4 *Wo* 2 $ms_{10}$ 5 *s*. The five genes within the brackets are in the positions indicated but the recombination values within each bracket are not known. While this linear order gives the best synthesis of the 61 recombination values, it also reveals several exceptions. The *aw ps* and *aw o* values which should be less than 10% are 40%, and *ps o* which should be 3% is 26%. Significant differences of more than 10% are also registered between sets of data for the same pair of genes; this is another indication of chromosomal changes. The findings presented here should furnish a framework for more critical examination of specific sections of this linkage group.


## Introduction

The linkage group of chromosome 2 was the first one discovered in the tomato. Hedrick and Booth (7) in 1907 showed that dwarf and pear were linked. This group was also the first one shown to be associated with a particular chromosome when Lesley (10) demonstrated that these genes are on the nucleolar chromosome. In recent years more mutants have been assigned to this group than to any of the other 11 chromosomes. The original linkage group consisted of the genes *d p o s* of Lindstrom (11) and MacArthur (12), and was known as linkage group No. 1. Now that most of the original linkage groups have been associated with their respective chromosomes, it is time to discontinue the dual numbering system. Therefore, the artificial system of assigning a Roman numeral based on order of discovery has been dropped in favor of the number system based on length of the pachytene chromosomes as proposed by Barton (1). Under this system linkage group 1 becomes chromosome group 2 since it is located on the second longest chromosome. Of the original four genes in this group, all except *o* give good monogenic segregations. The gene *o* is more difficult to classify, since it is responsible for the various fruit shapes from sphere, plum, calabash, pepper, to pear; and from the segregation ratios it appears that this locus may be a complex one. Several of the newer genes have lethal effects which disturb the monogenic segregations, but most accounts in the literature pay scant attention to these

[1]Manuscript received January 21, 1960.
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disturbances, and none of them until Butler (5) have pointed out the heterogeneity of some of the linkage values. It has been assumed that the chromosomes are all homologous but the heterogeneity values indicate that the chromosomes may contain inversions, duplications, or deficiencies. In this paper I shall present the results obtained from growing almost half a million plants, to give a map of the genes which will serve as a basis for precise studies of certain sections. These plants were not all grown in the same season, and at this stage in the investigation no allowance is made for seasonal effects on recombination values. In general the $P_1$ and $F_1$ plants were grown in the greenhouse, and the $F_2$'s were planted in the field. No temperature, rainfall, pH, or fertility records were kept although it was realized that such things might affect the recombination values. It was decided that in the absence of isogenic lines, no environmental control was necessary, because the recombination values are probably more affected by the genotypic background than by the environment.

## Description of the Characters

*d, Dwarf plants.*—The seedling plant has shorter hypocotyls and broader cotyledons, leaflets that are darker green, wrinkled, and sessile. The stems are usually shorter and thicker, making the adult plant a true dwarf, but numerous modifying genes make stature less reliable than the seedling characteristics. Most dwarf varieties are probably derived ultimately from the original "Laye Upright" mutant (Vilmorin-Andrieux (18)), which appeared in a French garden about 1850. Intercrosses of the various dwarf varieties have yielded only dwarf plants although these showed some differences in growth habit.

*$d^z$, Extreme dwarf.*—Hypocotyls very short, cotyledons short, dark, and strongly recurved. Plant is short, stocky, compact, and slow-growing, rarely exceeding 18 in. in height when full-grown. Lateral branches are infrequent so that most of the growth is confined to a single stem. The leaves are darker and more wrinkled than in dwarf, and the midribs are twisted. The flower stems and the whole inflorescence are shortened. This is a multiple allele of dwarf and is illustrated on page 281 of Rick and Butler (15).

*p, Pubescent or peach fruits.*—Denser and more persistent pubescence of the fruit epidermis imparting a dull appearance to the fruit surface in both green and ripe fruits. The texture of the fruit is less firm, and the locules are often partly empty as a result of the non-development of the cellular pulp which normally surrounds the seeds. Foliage is also more pubescent and in the seedling stage it often has a blue-gray cast.

*o, Ovate or pear-shaped fruits.*—The ovate fruits, or tomatoes which have a polar diameter which is at least 1½ times longer than the equatorial one, usually segregate as a recessive, but the final fruit shape is more complex. The pear, plum, pepper, and calabash shapes breed true in the original varieties and selections, but do not come true to shape in the $F_2$. For example, the cross of pear × round will give plum-shaped as well as pear-shaped

segregates in the $F_2$. While locule number and modifying genes may account for most of the exceptional segregations, there is some evidence that the *o* locus is a complex one.

*s, Compound inflorescence.*—In compound inflorescence the pedicel becomes much-branched to produce a globular mass of as many as 80 flowers in a single inflorescence. In the dominant simple inflorescence, the flower clusters are usually three internodes apart, whereas in the compound inflorescence, they are usually six internodes apart.

*aw, Without anthocyanin.*—This green-stem type was derived from the $F_1$ *L. esculentum* × *L. peruvianum* (Dennett and Larson (6)). It is phenotypically indistinguishable from many of the other anthocyanin genes.

*bk, Beaked fruits.*—The base of the style persists and becomes a pointed beaklike process on the stylar end of the fruit. The expression varies between genotypes and even between fruits on the same plant. The best expression occurs in two-loculed pear-shaped fruits.

*Cu, Curled leaves.*—In plants with curled leaves the petiole is much shortened and curled so that the leaves are closely applied to the central stem of the plant which is thinner than normal instead of being thickened as in the dwarf types. The plant has few branches, most growth being confined to the main stem which, if supported, will reach heights of 10 ft or more. This dominant gene, which is fully described by Young (20), is easily distinguished in the seedling stage as can be seen from Fig. 1.

*dv, Dwarf virescent.*—Cotyledons and true leaves are pale yellow-green at first, but change quickly to normal green. The growth rate, size of parts, and size of whole plant are usually less than normal, but some *dv* plants have normal growth rates after the early seedling stage.

*Lc, Locule diminution.*—The low number of locules per fruit (usually two) is dominant to the normal four- to six-loculed fruits. This character shows a great deal of variability even between fruits on the same plant, and is impossible to segregate in some $F_2$'s. Both Dennett and Larson (6) and Jenkins (8) suggest that locule number is a pleiotropic affect of the shape gene *o*.

*m, Mottled leaves.*—Cotyledons and leaves speckled with light yellow-green spots, rather unevenly distributed and tending to be somewhat elongate. Phenotype suggests the virus-borne mosaic disease but grafting experiments prove that *m* is cell-specific and not transmissible. Some combinations of *m* have twisted and deformed leaves and are unfruitful.

*Me, Mouse ears.*—In the homozygote the foliage is scanty, but the petioles are long, and the terminal segments are curled and shaped somewhat like the ear of a mouse. In the heterozygote the leaves are several times pinnately divided instead of once as in the normal $me^+$ type. Plants are illustrated in Rick and Harrison (16). The plants are spreading, much-branched, and difficult to train to a single stem. The seedling recognition is good as shown in Fig. 2.

*$ms_2$, Male-sterile 2.*—Anthers are pale and shrunken with no pollen in the mature anthers (Rick and Butler (15)).

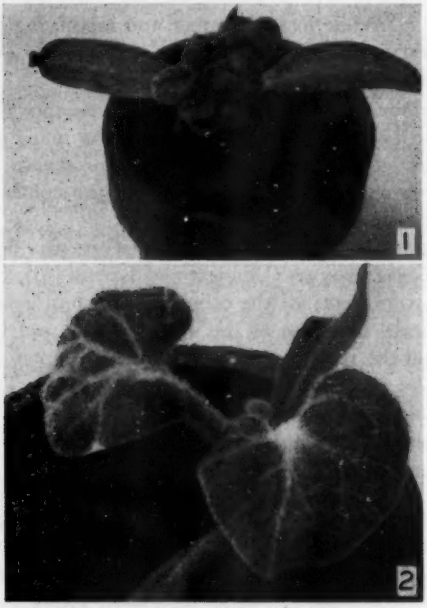


FIG. 1. Curled mutant in the seedling stage.
FIG. 2. Woolly, mouse-eared seedling, *Wo wo Me Me.*

*ne, Necrotic plants.*—Plants show a progressive breaking-down and death of the tissue without the presence of any pathogens (Langford (9)). The ***ne ne*** plants manifest this effect only in the presence of the gene $Cf_2$.

*ps, Position sterile.*—The petals are so tightly bound in the dorsal grooves of the anthers into which they normally fold in the bud that they do not ordinarily unfurl. The mature flower resembles a vase. Stomia of the anthers usually do not open during the life of the flower, thereby preventing the selfing by the pollen, which is of normal viability.

*ro, Rosette.*—Hypocotyl less than half normal length, and very thin. All leaves emerge from the region just above the cotyledons, which are persistent for a long time. Axillary branching suppressed, and branching of the roots also reduced so that in the seedling stage there is only a single tap root. No flowers are produced. The germination of the homozygote is reduced; only 50–80% of the expected *ro ro* plants appear.

*Wo, Woolly plants.*—All vegetative parts, including the hypocotyl and the epidermis of the fruit, are densely tomentose. The long hairs are more abundant, branched, and stellate, but no longer than normal. Woolly plants can be detected even in the gene combinations *Wo wo H H* and *Wo wo hl hl.* The homozygote is usually lethal, rarely germinating, but when it does it dies in the cotyledon stage before the expansion of the plumule. Such seedlings have abnormally thick and shortened hypocotyls and cotyledons. When selfed, woolly gives a ration of two woolly to one normal.

*Wo*$^{m}$, *Morgan's woolly.*—Similar in action and allelic to *Wo* but less extreme. Homozygotes are viable, all three genotypes being distinguished, especially under the climatic conditions of California. At Toronto, the *Wo*$^{m}$ *wo* plants are difficult to differentiate from the *wo wo* plants in the seedling stage.

*Wo*$^{v}$, *Van Wert's woolly.*—Similar in action and allelic to *Wo* but more extreme. The heterozygote is as extreme in pubescence as the *Wo Wo* plants, the leaves are curled downwards, the internodes and rachis are longer than usual making the plant appear spindly. The fertility is lower than in the other two dominant alleles. The homozygote is lethal.

## The Monogenic Ratios

In Table I it will be seen that 23 of the 78 chi-square values are large enough to indicate significant departures from expected and need an explanation.

TABLE I

The segregation numbers and recombination values of *D* with the other genes of chromosome 2

| Test gene | Phase | *D X* | *D x* | *d X* | *d x* | $\chi^2$ *D* | $\chi^2$ *x* | Recombination, % ± S.E. |
|---|---|---|---|---|---|---|---|---|
| *Dv* | $F_2$R | 5187 | 1980 | 2239 | 5 | 6.7 | 76.6 | 5.4±1.0 |
| | B R | 1 | 286 | 254 | 6 | 1.3 | 2.4 | 1.3±0.1 |
| *M*(*Wo*) | $F_2$C | 6420 | 95 | 80 | 1833 | 19.7 | 16.3 | 2.1±0.2 |
| (*wo*) | $F_2$C | 12862 | 200 | 225 | 4040 | 2.6 | 1.4 | 2.4±0.1 |
| (*Wo*) | $F_2$R | 2262 | 992 | 938 | 47 | 7.0 | 0.5 | 22.3±0.7 |
| (*wo*) | $F_2$R | 1609 | 735 | 802 | 21 | 1.6 | 2.2 | 16.3±0.7 |
| | B C | 1367 | 6 | 19 | 1250 | 4.1 | 6.4 | 0.9±0.6 |
| *P* | $F_2$C | 3570 | 148 | 106 | 1018 | 8.2 | 2.2 | 5.4±0.3 |
| | $F_2$R | 414 | 174 | 168 | 0 | 3.1 | 1.6 | 3.0±3.6 |
| | B C | 760 | 22 | 33 | 711 | 0.9 | 2.4 | 3.6±0.5 |
| *Ps* | $F_2$C | 650 | 39 | 62 | 181 | 0.6 | 1.0 | 11.3±1.0 |
| | $F_2$R | 606 | 260 | 250 | 9 | 2.3 | 0.7 | 19.5±2.5 |
| | B C | 45 | 4 | 3 | 46 | 0.0 | 0.0 | 7.1±2.6 |
| | B R | 22 | 188 | 175 | 16 | 0.9 | 0.1 | 9.5±1.5 |
| *O* | $F_2$C | 1621 | 251 | 167 | 470 | 0.2 | 18.7 | 17.4±0.8 |
| | $F_2$R | 730 | 305 | 286 | 15 | 4.3 | 0.9 | 23.3±2.6 |
| | B C | 81 | 11 | 10 | 67 | 1.3 | 1.0 | 12.4±2.5 |
| | B R | 50 | 256 | 294 | 43 | 1.5 | 3.1 | 14.5±1.4 |
| *Aw* | $F_2$C | 7332 | 696 | 683 | 2025 | 0.3 | 0.7 | 13.9±0.4 |
| | $F_2$R | 8020 | 3677 | 3761 | 236 | 1.8 | 0.0 | 24.3±0.8 |
| | B C | 2221 | 316 | 314 | 2111 | 2.5 | 2.4 | 12.7±0.4 |
| | B R | 41 | 190 | 193 | 51 | 0.4 | 0.1 | 19.4±1.6 |
| *Me* | $F_2$C | 574 | 70 | 76 | 84 | 11.2 | 14.7 | 23.1±1.7 |
| | B C | 384 | 102 | 97 | 305 | 7.9 | 6.2 | 22.4±1.4 |
| *Cu* | $F_2$C | 1536 | 222 | 343 | 249 | 0.0 | 30.8 | 29.2±1.1 |
| | B C | 118 | 36 | 37 | 104 | 0.6 | 0.8 | 24.7±2.5 |
| *Bk* | $F_2$C | 1081 | 196 | 214 | 273 | 6.4 | 2.4 | 25.7±1.4 |
| | $F_2$R | 187 | 70 | 71 | 3 | 1.2 | 1.5 | 22.4±5.1 |
| *Ro* | $F_2$R | 2981 | 736 | 1186 | 53 | 0.0 | 231.4 | 27.3±1.3 |
| $Ms_{10}$ | $F_2$C | 197 | 21 | 45 | 15 | 1.7 | 3.5 | 34.7±3.7 |
| | $F_2$R | 866 | 361 | 346 | 23 | 3.0 | 0.8 | 25.9±2.3 |
| *S* | $F_2$C | 2001 | 435 | 457 | 391 | 1.2 | 0.0 | 32.0±1.0 |
| | $F_2$R | 539 | 215 | 221 | 20 | 0.3 | 1.0 | 30.0±2.8 |
| | B C | 67 | 25 | 26 | 51 | 1.3 | 1.7 | 30.2±3.5 |
| | B R | 57 | 98 | 149 | 30 | 1.6 | 18.2 | 26.0±2.4 |
| *Wo* | $F_2$C | 5044 | 2000 | 1377 | 1308 | 35.0 | 1.9 | 37.9±0.7 |
| | $F_2$R | 4985 | 2179 | 1297 | 169 | 295.5 | 97.2 | 33.4±0.9 |
| | B C | 318 | 132 | 115 | 270 | 5.1 | 1.2 | 29.6±1.6 |
| | B R | 406 | 1078 | 973 | 343 | 10.1 | 0.6 | 26.7±0.8 |

NOTE: C = coupling, R = repulsion, *X* = dominant allele of tester gene, *x* = recessive allele of tester gene.

Of the 23 significant chi-squares 13 of them are for the *d* gene and in all cases are the result of a deficiency of dwarf plants. The first pair, *d dv*, have a shortage of *dv* plants which usually show poorer germination and have plants which are weaker in the seedling stage. The shortage of *dv*'s caused an excess of *D*'s and this accounts for the chi-square for *d* of 6.7. The next three significant chi-squares are caused by the gene woolly which being lethal in the homozygote distorts the *m d* segregations. The deficiencies of dwarf and mottled plants accounts for the next three large chi-squares. The 18.7 chi-square for *o* is the result of difficulty of classification; some 70 plants were probably misclassified. Both sets of data involving *Me d* show departures from expected; this effect of the *Me* gene has been noted before, and will be discussed later. In the case of *Cu* the departure is the result of an excess of curled plants probably as a result of the same mechanism which distorts the *Me* data. The 231.4 chi-square is brought about by the failure of the *ro* plants to germinate. In the *d s* backcross there is an excess of *S* plants which may be the result of misclassification as in certain backgrounds the expression of *s* is not good. The remainder of the large chi-squares occur in crosses in which woolly is segregating.

In Table II, 10 of the 22 chi-square values for *aw*, and 11 of the ones for the other genes, show significant departures from the expected. The departures from expected in the *aw* ratio are the result of interaction with *dv*, *Me*, *Cu*, *ro*, and *Wo*, the same genes which caused the large chi-squares in Table I. In the case of *aw p* there is an excess of *aw P* segregates; these 70 excess plants may be the result of contamination with *a* which is phenotypically identical. If this is so, then the recombination value of 13.5 should be smaller, much

TABLE II

The segregation ratios and recombination percentage between *Aw* and other chromosome 2 genes

| Gene | Phase | *Aw X* | *Aw x* | *aw X* | *aw x* | $\chi^2$ *Aw* | $\chi^2$ *x* | Recombination |
|---|---|---|---|---|---|---|---|---|
| *Dv* | $F_2$C | 966 | 96 | 149 | 239 | 2.4 | 2.8 | 18.2±1.1 |
| | $F_2$R | 2990 | 1035 | 1177 | 32 | 10.1 | 59.5 | 18.9±4.0 |
| *M* | $F_2$C | 7882 | 618 | 799 | 1919 | 3.6 | 34.0 | 13.9±0.4 |
| | $F_2$R | 5562 | 2329 | 2603 | 65 | 0.4 | 30.4 | 16.6±0.9 |
| | B C | 1568 | 221 | 270 | 1440 | 1.8 | 8.9 | 14.0±0.6 |
| *P* | $F_2$C | 629 | 34 | 105 | 181 | 13.4 | 2.8 | 13.5±1.2 |
| | B C | 257 | 20 | 29 | 268 | 0.7 | 0.0 | 8.5±1.1 |
| *Ps* | $F_2$C | 134 | 43 | 49 | 20 | 1.2 | 0.0 | 46.5±4.5 |
| | $F_2$R | 540 | 217 | 209 | 34 | 0.3 | 0.0 | 37.8±2.7 |
| *O* | $F_2$C | 266 | 74 | 79 | 47 | 0.9 | 0.2 | 39.5±2.8 |
| *Me* | $F_2$C | 320 | 44 | 35 | 56 | 6.1 | 2.2 | 20.9±2.2 |
| | B C | 434 | 48 | 50 | 365 | 5.0 | 5.6 | 10.9±1.0 |
| *Cu* | $F_2$C | 1814 | 85 | 87 | 390 | 30.7 | 31.8 | 8.2±0.6 |
| | B C | 146 | 16 | 8 | 125 | 2.8 | 0.6 | 8.1±1.6 |
| *Ro* | $F_2$R | 8878 | 1975 | 4152 | 65 | 71.5 | 171.4 | 17.9±0.8 |
| $Ms_{10}$ | $F_2$R | 248 | 94 | 104 | 9 | 0.0 | 0.8 | 30.0±4.1 |
| *S* | $F_2$C | 410 | 101 | 108 | 73 | 0.5 | 0.0 | 36.3±2.4 |
| | $F_2$R | 142 | 41 | 48 | 4 | 1.0 | 4.3 | 32.9±5.7 |
| *Wo* | $F_2$C | 3047 | 640 | 477 | 1006 | 37.4 | 5.2 | 22.2±0.7 |
| | $F_2$R | 2116 | 1108 | 374 | 46 | 352.8 | 4.5 | 30.4±1.5 |
| | B C | 148 | 19 | 28 | 92 | 7.7 | 19.6 | 16.4±2.1 |
| | B R | 119 | 511 | 467 | 70 | 7.4 | 0.0 | 16.2±1.0 |

closer to the 8.5% of the backcross. The significant values in the second column of chi-squares are all the results of the genes mentioned in connection with Table I.

In Table III all the chi-square values for *s* are all right, except in the case of *S Wo* $F_2$ where the lethal *Wo Wo* caused a deficiency of the linked *S* segregates. Incidentally, the *Wo* chi-squares are calculated on the basis of 2:1 instead of 3:1, and thus give a good fit when the dominant homozygote class is missing. Of the genes paired with *s* in this table, only two have significant chi-squares; these are *bk* and *o*. Both these genes are difficult to classify in certain backgrounds, so no doubt this is the cause of the large chi-squares. Of the 13 chi-square values for *p*, only one is significant at the .05 level, and this can be attributed to chance. The other three significant chi-squares are in segregations which contain the genes *Wo*, *o*, or *dv*, all of which have been noted before as giving disturbed segregations.

TABLE III

The segregation numbers and recombination values of *S* and *P* with the other genes of chromosome 2

| Gene pair | Phase | *a* | *b* | *c* | *d* | $\chi^2_1$ | $\chi^2_2$ | Recombination |
|---|---|---|---|---|---|---|---|---|
| *S Bk* | $F_2$C | 1587 | 123 | 87 | 505 | 0.6 | 6.6 | 9.2±0.6 |
| *S Ps* | $F_2$R | 270 | 130 | 148 | 6 | 2.3 | 0.1 | 19.5±4.0 |
| *S O* | $F_2$C | 1853 | 377 | 290 | 478 | 0.6 | 19.8 | 24.5±0.9 |
| | $F_2$R | 216 | 97 | 93 | 15 | 0.1 | 0.7 | 35.8±4.2 |
| | B C | 192 | 53 | 41 | 189 | 0.5 | 0.2 | 19.8±1.5 |
| | B R | 37 | 125 | 124 | 36 | 0.0 | 0.0 | 32.9±3.0 |
| *S Wo* | $F_2$C | 866 | 143 | 130 | 308 | 21.4 | 3.1 | 19.2±1.2 |
| | B C | 86 | 5 | 7 | 71 | 0.3 | 0.9 | 7.1±6.2 |
| *S P* | $F_2$C | 1497 | 311 | 271 | 318 | 0.2 | 2.0 | 27.9±1.1 |
| | $F_2$R | 348 | 133 | 149 | 13 | 0.0 | 1.8 | 30.0±3.5 |
| | B C | 68 | 25 | 24 | 52 | 1.7 | 1.3 | 29.0±3.4 |
| *S M* | $F_2$C | 149 | 20 | 30 | 29 | 0.1 | 1.5 | 25.4±3.3 |
| | $F_2$R | 284 | 125 | 128 | 4 | 0.1 | 0.4 | 18.1±4.1 |
| *S Dv* | $F_2$C | 248 | 58 | 55 | 56 | 0.6 | 1.2 | 30.8±2.7 |
| | $F_2$R | 161 | 83 | 74 | 9 | 0.0 | 1.7 | 30.4±4.9 |
| *P M* | $F_2$C | 1254 | 55 | 56 | 335 | 3.4 | 3.6 | 6.7±0.6 |
| | B C | 203 | 8 | 9 | 208 | 0.1 | 0.0 | 4.0±0.9 |
| *P Dv* | $F_2$R | 711 | 281 | 314 | 6 | 0.3 | 6.8 | 15.0±2.7 |
| *P Ps* | $F_2$R | 231 | 104 | 96 | 1 | 1.5 | 0.1 | 10.5±4.7 |
| | B R | 8 | 20 | 20 | 3 | 0.5 | 0.5 | 21.5±5.7 |
| *P O* | $F_2$C | 1699 | 221 | 163 | 489 | 0.2 | 9.3 | 16.6±0.8 |
| | $F_2$R | 422 | 201 | 207 | 10 | 0.3 | 0.0 | 21.2±3.2 |
| *P Me* | B C | 58 | 7 | 13 | 60 | 0.5 | 0.1 | 14.5±2.9 |
| *P Bk* | $F_2$C | 1029 | 192 | 197 | 260 | 4.5 | 3.4 | 25.6±1.3 |
| | $F_2$R | 179 | 72 | 79 | 1 | 0.1 | 0.6 | 12.2±5.4 |
| *P Wo* | $F_2$C | 802 | 233 | 117 | 213 | 0.5 | 0.3 | 26.8±1.4 |
| | B C | 156 | 36 | 42 | 116 | 3.3 | 6.0 | 22.3±2.2 |
| | B R | 49 | 116 | 124 | 38 | 0.0 | 1.0 | 26.6±2.4 |

In Table IV, half of the chi-squares for the gene *m* show disturbed segregations and in all cases these are the result of a lack of *m* segregates. The other large chi-squares in the first half of the table are from *dv*, *ro*, and *Wo*. In the second part of the table where the various genes are paired with woolly, it will be seen that the chi-squares for *Wo* fit the 2:1 expected except in the case of *Wo ro*, where the lack of woolly rosettes has disturbed the segregation.

TABLE IV

Segregation ratios and recombination values of the remaining chromosome 2 genes

| Gene pair | Phase | *a* | *b* | *c* | *d* | $\chi^2_1$ | $\chi^2_2$ | Recombination |
|---|---|---|---|---|---|---|---|---|
| 1 2 | | | | | | | | |
| *M Dv* | $F_2$R | 3735 | 1477 | 1574 | 6 | 10.9 | 36.3 | 6.9±1.2 |
| | $F_2$R | 542 | 170 | 146 | 35 | 10.7 | 2.0 | 46.1±2.6 |
| *M Ps* | $F_2$R | 41 | 14 | 25 | 6 | 5.6 | 0.1 | 44.9±8.3 |
| *M* $Ms_{10}$ | $F_2$R | 290 | 107 | 76 | 10 | 13.3 | 0.2 | 35.7±3.9 |
| *M Ro* | $F_2$R | 303 | 84 | 136 | 0 | 0.3 | 22.3 | 11.3±4.3 |
| *M O* | $F_2$C | 203 | 46 | 35 | 35 | 1.6 | 0.0 | 30.7±3.1 |
| | $F_2$R | 177 | 104 | 89 | 9 | 0.1 | 4.7 | 26.7±4.7 |
| *M Bk* | $F_2$R | 194 | 88 | 94 | 4 | 0.1 | 0.1 | 20.5±4.8 |
| *M Me* | B C | 257 | 93 | 72 | 241 | 2.1 | 0.0 | 24.9±1.7 |
| *M Cu* | $F_2$C | 287 | 42 | 43 | 51 | 1.7 | 2.0 | 24.2±2.4 |
| | B C | 107 | 32 | 37 | 93 | 0.3 | 1.3 | 25.6±2.6 |
| *M Wo* | $F_2$C | 5193 | 1258 | 904 | 1203 | 0.7 | 80.7 | 28.2±0.6 |
| | $F_2$R | 3497 | 1979 | 834 | 143 | 334.6 | 0.5 | 33.5±1.0 |
| | B C | 371 | 243 | 185 | 348 | 5.7 | 1.3 | 37.3±1.4 |
| | B R | 130 | 361 | 324 | 86 | 7.3 | 0.1 | 24.0±1.4 |
| *Wo Dv* | $F_2$C | 1612 | 266 | 484 | 423 | 0.7 | 0.1 | 28.6±1.0 |
| | B C | 66 | 9 | 21 | 36 | 2.5 | 13.4 | 22.7±3.6 |
| *Wo Ps* | B C | 173 | 27 | 24 | 177 | 0.0 | 0.0 | 12.7±1.6 |
| *Wo* $Ms_{10}$ | $F_2$C | 384 | 2 | 12 | 158 | 1.9 | 4.2 | 2.5±0.6 |
| *Wo Ro* | $F_2$C | 2260 | 144 | 763 | 342 | 5.4 | 232.7 | 25.6±0.9 |
| *Wo O* | $F_2$C | 591 | 81 | 152 | 185 | 0.0 | 1.0 | 23.4±1.5 |
| | B C | 78 | 13 | 13 | 65 | 1.0 | 1.0 | 15.3±2.7 |
| *Wo Bk* | $F_2$C | 425 | 45 | 67 | 193 | 1.7 | 22.5 | 14.6±1.4 |
| *Wo Me* | $F_2$R | 252 | 24 | 141 | 1 | 0.1 | 80.6 | 18.4±4.7 |
| | B R | 69 | 465 | 422 | 65 | 2.2 | 1.5 | 13.1±1.0 |
| *Wo Cu* | B R | 0 | 124 | 144 | 1 | 1.6 | 1.3 | 0.4±0.4 |
| *O Dv* | $F_2$R | 157 | 48 | 68 | 4 | 0.1 | 5.7 | 28.0±5.4 |
| *O Ps* | $F_2$R | 259 | 107 | 119 | 8 | 0.1 | 0.7 | 26.1±4.1 |
| *O Bk* | $F_2$C | 1483 | 203 | 204 | 457 | 12.5 | 12.5 | 18.1±1.0 |
| *Ps Bk* | $F_2$R | 93 | 28 | 29 | 0 | 2.5 | 2.5 | 22.3±7.8 |
| *Ps* $Ms_{10}$ | $F_2$R | 173 | 36 | 60 | 0 | 1.0 | 19.4 | 15.0±3.4 |
| *Ps Dv* | $F_2$R | 180 | 101 | 96 | 0 | 0.0 | 0.0 | 9.3±5.0 |
| *Dv Bk* | $F_2$R | 166 | 59 | 46 | 6 | 5.7 | 0.3 | 36.1±5.1 |
| *Dv Cu* | $F_2$C | 365 | 21 | 19 | 59 | 16.6 | 14.9 | 10.6±1.5 |
| *Dv Ro* | $F_2$R | 862 | 187 | 407 | 8 | 8.7 | 106.5 | 20.1±2.5 |

The ratios of *dv* and *ro* again show a deficiency of the recessive class, and *bk* has a large chi-square partly because of the linkage effect of *Wo* and partly because of difficulty of classification. *Me* has a greatly disturbed ratio characterized by a great deficiency of *me* plants, the ratio being 15.7:1 instead of the expected 3:1. With a recombination value of 10% between *Wo* and *Me* and death of all *Wo Wo* a ratio of 14.8:1 would be expected so that linkage of 10% or closer would explain the paucity of *me* plants. The $ms_{10}$ plants are difficult to classify at Toronto, and under cool summer conditions a male sterile may appear normal. This misclassification is probably the cause for the lack of $ms_{10}$ segregates in the *Ps* $Ms_{10}$ crosses. The large chi-squares for *dv* and *ro* are again the result of the low viability of these genes while the disturbed ratio of *Cu* in this case is the effect of *Wo* which was also segregating in this cross.

From the foregoing it is apparent that many of the disturbed ratios are the result of poor germination of mutants such as *ro* and *dv*; whereas others stem

from the misclassification of mutants such as *o*, *bk*, and $ms_{10}$. There are also a large number of ratios which are disturbed because of linkage of the gene with *Wo*, and the consequent elimination of the mutant along with the 25% of the plants which are *Wo Wo*. For genes closely linked with *Wo*, and in the repulsion phase, this may disturb the monogenic ratio to such an extent that it becomes 15 or even 20:1 instead of 3:1. Once the recombination value between *Wo* and any gene is known, then the observed ratio can be tested against the ratio expected. There is one complication to this procedure, namely, that in some of the more recent crosses the ratio is no longer 2:1. As I pointed out previously (Butler (4)), from 1933 until 1952, the $P_1$ and $F_2$ ratios for *Wo* were always 2:1 but in cross 5221 (see Table V) it is obviously not a 2:1 ratio. It will be seen from Table V that of the seven listed crosses, four fit the 2:1 ratio, and two fit neither the 2:1 nor 3:1 ratio. In cross 5221, the first results did fit a 3:1 ratio and it was thought that a lethal had been linked with *Wo*, and that a rare crossover had given a viable homozygous

TABLE V

Heterogeneity of woolly monogenic segregations

| Cross | *Wo* | *wo* | *wo* expected | | $\chi^2$ 2:1 | $\chi^2$ 3:1 |
|---|---|---|---|---|---|---|
| | | | 2:1 | 3:1 | | |
| 5221 | 2963 | 847 | 1276 | 957 | 210.3 | 15.5 |
| 5321 | 1338 | 696 | 678 | 508 | 0.7 | 92.2 |
| 5405 | 507 | 167 | 225 | 168 | 22.2 | 0.0 |
| 5719 | 500 | 229 | 243 | 182 | 1.2 | 16.0 |
| 5851 | 785 | 353 | 379 | 285 | 2.7 | 22.0 |
| 5818 | 594 | 299 | 298 | 223 | 0.0 | 34.3 |
| 5704 | 6097 | 2461 | 2853 | 2139 | 80.7 | 64.6 |

*Wo Wo*. Further investigation, however, showed that the $F_2$ contained no homozygous *Wo*, and that the ratio was not 3:1 but almost 4:1. There is no significant heterogeneity between lots, and it is difficult to account for these results by differential pollen or ovule fertility. The simplest explanation is that a duplication has taken place and that *Wo Wo/wo* is viable. If we make this assumption and also assume that the two *Wo* loci are about 25% apart, this would give the ratios obtained. This would also account for some of the anomalous recombination values to be discussed in the next sections. It is not suggested that this is the only explanation which will account for the observed ratios and recombination values, but it does form a provisional hypothesis which can be tested by future crosses.

Additional evidence in favor of the duplication hypothesis comes from the monogenic ratios of *aw* in crosses derived from this same stock. In one case 15 fruits produced 362 *Aw* : 76 *aw*, and there was no significant heterogeneity between fruits. This ratio could also be explained by duplication and a crossover value of less than 20%. However, since we do not know anything about the possible position effect of these genes, this must also await further crosses.

## The Linkage Values

The map as given by MacArthur (12, 13, 14) is *D*-4-*P*-10-*O*-20-*S*, with more variability of the linkage values shown in the later paper than in the two former ones. Young and MacArthur (19) placed *Wo*, *m*, *bk*, and *lc* in this group but did not publish the data. Langford (9) placed *ne* in this group, and Butler (2) summarized the data for these genes. Robinson and Rick (17) placed *dv* in this group and Butler (3) placed *ro*. Dennett and Larson (6) placed *aw* in this group and suggested a new classification of the *o* gene based on its association with locule number. Recently, Rick and Harrison (16) have shown that *Cu* and *d* are linked with 35–39% crossover, and *Me* and *d* with 27–34% recombination. They also suggest that *ff*, a new mutant, is in this group.

Backcrosses involving various groups of three or more of these genes have been made but because of the closeness of the linkage, or the poor viability of certain combinations, or possibly chromosome abnormalities, the multiple recombination classes do not always give a consistent order. Therefore the bulk of the onus in determining linear order will have to rest on the $F_2$'s.

Examination of the linkage data in Tables I–VIII indicates that the relative positions of most of the genes are constant but that some seem to occupy two different positions in the chromosome. Using the data from Tables I, VI, and VII, we get the order *dv m d p ps o aw Me Cu bk ro* $ms_{10}$ *Wo S*, but some of the loci are so close together that large backcrosses would be necessary to get sufficient resolution to be sure of the order. Using the data from Table II we know now that *aw* is near the center of this group and also on which side of the center each gene should lie; we find that most of the data are compatible with the order obtained from Table I. There are minor exceptions, in that *Cu* and *Me* are reversed, but since these two loci are close together and the data are meager, this is not important. The data for the *aw s* distance puts *s* farther from *aw* than it is from *d*, whereas it should be closer. Woolly is closer to *aw* than it is to *d* but the four values are not consistent; in the two backcrosses the recombination value is 16% but the *aw* segregation is disturbed in both cases, and the *Wo* ratio is disturbed in the coupling phase. The coupling $F_2$ has a significantly higher recombination value, and again has disturbed monogenic values. The repulsion $F_2$ has a recombination value which is significantly higher than any of the others, and the monogenic ratio for *aw* is greatly disturbed, there being 491 less *aw* plants than expected. The disturbed segregations of *wo aw*, and *Cu Wo aw* crosses, all point to a chromosomal abnormality in this region.

The major difference between Tables I and II is in the *aw ps o* values. These three genes would be expected to lie within 5 to 10% of one another; instead *o* is 39% and *ps* is 40% away from *aw*. This means either that *ps* and *o* should have been put on the other side of dwarf in the first place, or that *ps o* or *aw* have been inverted in the second place. All the other evidence shows that *o* is between *p* and *s*, and Table IV shows that *ps* is a long distance from *m*, so it looks as though an inversion is involved in the *aw* data. The distance

from *aw* to *dv m p* has remained the same, but the distance from *aw* to *o* and *ps* has greatly increased. None of the data are ideal for pinning down the inversion because each set of data may be a mixture of recombination types. It appears that the segment containing *aw Wo* and *Me* has been duplicated and that this is the reason for the disturbed monogenic ratios of these genes and for the anomalous linkage ratios with *o* and *ps*. For instance, if *Aw* is duplicated at the *s* end and the two *Aw*'s are 40 units apart, then the cross *Aw Aw* × *aw* would give a ratio of approximately 9:1 instead of 3:1; and the recombination values for *ps o* would be as in Table II. Since some of the values in Table II are calculated with the *aw* in the normal position and the rest with it at the end of the chromosome, some values are as expected from the results in Table I, whereas others are not.

In Table III the recombination values for genes paired with *s* are given. Examination of these values shows that all are consistent with the values and positions determined in Table I except that *ps* is on the opposite side of *o* and *m* is a little closer to *s* than expected. The recombination values for *p* are given in the same table, and these fit into the same general pattern except for *Me* which is closer to *p* than expected.

In Table IV, we have cases in which *m* is used as the tester gene and some anomalous values are obtained. The genes *m* and *dv* are 7 units apart in coupling and 46 units in repulsion. *Ps* is 45 units from *m* instead of less than 20, and *ro* is 11 units away instead of 28 units. The order for this set is *dv m ro bk Me Cu o Wo* $ms_{10}$ *ps dv*. The change in position of *ro* can be explained by the large errors involved as a result of the poor germination of *ro* genotypes. The *dv* and *ps* results could be explained by an inversion, but when further crosses are made a simpler explanation may be found for this. In the same table the recombinations with *Wo* are given, and these present no special problems. *Ps* is 13 units from *Wo* which is the right distance for *ps* in either a medial or a distal position. *Ro* is closer to *d* than indicated in Table I. The latter part of Table IV lists cases where *o ps* and *dv* are used as the tester genes. The gene *o* is equidistant from *dv* and *ps*, which again indicates that *ps* is in the distal instead of the medial position, though it is impossible to say whether *dv* is on the same side as *ps* or in its normal position on the opposite side. The *ps bk* and $ms_{10}$ values are the ones expected for *ps* in the distal position, but the *ps dv* is a close linkage indicating that *dv* is also at this end of the chromosome. The recombination values for *dv* with *bk* and *ro* are the ones expected, but the one for *dv* with *Cu* is much too close. Since it is difficult to classify *Cu dv* plants this may be the result of misclassification.

The backcross data given in Tables VI–VIII show that the order of these genes is *m d aw Me Wo*, *d p aw*, and *m d aw Cu Wo*. Unfortunately these genes are the ones which have been fairly consistent in all the tables, so these data do not furnish any new evidence beyond confirming the order of these genes. In Table VI it will be observed that although the chi-squares for *m* are not significant, in every case there is an excess of wild type genes. This is especially noticeable in the two classes of recombinants between *m* and *d* in

TABLE VI

Segregation in backcrosses $\frac{M\ D\ Aw}{m\ d\ aw} \times m\ d\ aw$

| Phenotype | Lot 1 | Lot 2 | Lot 3 | Recombinants | |
|---|---|---|---|---|---|
| $\frac{M\ D\ Aw}{m\ d\ aw}$ | 464<br>428 | 192<br>175 | 254<br>233 | $a_0$ | 1745 |
| $M \mid d\ aw$<br>$m \mid D\ Aw$ | 7<br>1 | 1<br>1 | 2<br>1 | $a_1$ | 13 |
| $M\ D \mid aw$<br>$m\ d \mid Aw$ | 54<br>40 | 41<br>34 | 53<br>45 | $a_2$ | 267 |
| $M \mid d \mid Aw$<br>$m \mid D \mid aw$ | 3<br>0 | 0<br>0 | 0<br>0 | $a_{12}$ | 3 |
| Total | 997 | 444 | 587 | | 2028 |
| $\chi^2_M$<br>$\chi^2_D$ | 3.49<br>1.69 | 1.30<br>1.30 | 1.43<br>1.43 | $y_1$<br>$y_2$ | 0.91%<br>15.5 % |
| $\chi^2_{Aw}$ | 0.36 | 0.23 | 0.29 | | |

TABLE VII

Segregation data for the backcross $\frac{M\ D\ Aw\ Me\ wo}{m\ d\ aw\ me\ Wo} \times m\ d\ aw\ me\ wo$

| Phenotype | Number | Gene | Ratio | $\chi^2$ | Recombination | |
|---|---|---|---|---|---|---|
| $M\ D\ Aw\ Me\ wo$<br>$m\ d\ aw\ me\ Wo$ | 215<br>196 | *Wo* | 289:300 | 0.21 | $y_1$ | 0.5% |
| $M \mid d\ aw\ me\ Wo$<br>$m \mid D\ Aw\ Me\ wo$ | 1<br>2 | *Aw* | 300:289 | 0.21 | $y_2$ | 16.6% |
| $M\ D \mid aw\ me\ Wo$<br>$m\ d \mid Aw\ Me\ wo$ | 46<br>37 | *Me* | 298:291 | 0.08 | $y_3$ | 11.2% |
| $M\ D\ Aw \mid me\ Wo$<br>$m\ d\ aw \mid Me\ wo$ | 30<br>28 | *D* | 310:279 | 1.63 | $y_4$ | 4.4% |
| $M\ D\ Aw\ Me \mid Wo$<br>$m\ d\ aw\ me \mid wo$ | 9<br>10 | *M* | 309:280 | 1.43 | | |
| $M\ D \mid aw \mid Me\ wo$<br>$m\ d \mid Aw \mid me\ Wo$ | 4<br>4 | | | | | |
| $M\ D \mid aw\ me \mid wo$<br>$m\ d \mid Aw\ Me \mid Wo$ | 4<br>3 | | | | | |
| | 589 | | | | | |

lot 1. The segregations 7:1 and 3:0 indicate either that the *m d* combination is sometimes classified as *M d* because of the darker green cotyledons of dwarf, or that *m* is less viable in all combinations. The recombination values indicate tight linkage between *m* and *d* and the number of double crossovers is just as expected although it would look better if they were more evenly divided between the six positions in the three lots. In Table VII the recombination values for positions 1 and 2 ($y_1$ and $y_2$) are similar to those given in Table VI,

TABLE VIII

Segregation data for the backcross $\frac{M\ D\ Aw\ Cu\ wo}{m\ d\ aw\ cu\ Wo} \times m\ d\ aw\ cu\ wo$

| Phenotype | Number | Gene | Ratio | $\chi^2$ | Recombination | |
|---|---|---|---|---|---|---|
| *M D Aw Cu wo* / *m d aw cu Wo* | 106 / 89 | *Wo* | 126:143 | 1.08 | | |
| | | | | | $y_1$ | 0.4% |
| *m* \| *D Aw Cu wo* / *M* \| *d aw cu Wo* | 0 / 1 | *Cu* | 142:127 | 0.84 | | |
| | | | | | $y_2$ | 21.2% |
| *m d* \| *Aw Cu wo* / *M D* \| *aw cu Wo* | 28 / 23 | *Aw* | 147:122 | 2.32 | | |
| | | | | | $y_3$ | 7.8% |
| *m d aw* \| *Cu wo* / *M D Aw* \| *cu Wo* | 7 / 9 | *D* | 140:129 | 0.45 | | |
| | | | | | $y_4$ | 0.4% |
| *m d aw cu* \| *wo* / *M D Aw Cu* \| *Wo* | 0 / 0 | *M* | 141:128 | 0.63 | | |
| *m d* \| *Aw* \| *cu Wo* / *M D* \| *aw* \| *Cu wo* | 4 / 1 | | | | | |
| *m d* \| *Aw Cu* \| *Wo* / *M D* \| *aw cu* \| *wo* | 0 / 1 | | | | | |
| | 269 | | | | | |

and $y_3$ is also similar to the B.C. values given for *aw Me* in Table II but it is only half the value obtained for $F_2$ C in the same table. The $y_4$ value of 4.4% is certainly not comparable with the values 18.4 and 13.1% given in Table IV, and this fact combined with the other exceptions points again to the assumption that the *Wo* locus occurs in two different positions. Rick and Harrison (16) also show a close linkage between *Me* and $Wo^m$. Table VIII gives comparable data for the *Cu* gene, and again the $y_1$, $y_2$, and $y_3$ values are comparable with those given in other tables. The *Cu Wo* distance of 0.4% indicates that this gene is even closer to *Wo* than is *Me*.

In order to summarize the recombination values, Table IX has been constructed. This table shows the most likely value of each recombination percentage with due regard to errors and disturbances in monogenic ratios. If this table is properly arranged and there is only one true linear order for these genes, then values should increase from left to right along each row, and decrease from top to bottom in each column. The smallest values should occur on the pivotal diagonal, and it is unfortunate that many of these values are missing. It is easy to see that there are many exceptions to these rules, yet no other arrangement tried gave better results. It is also obvious that the worst exceptions occur in *ps* and *o*, especially *aw ps*, *aw o*, and *ps o*. All three of these values are obtained from the same cross, which evidently possessed a different chromosome arrangement. It should also be noted that for certain combinations, two different recombination values are given, indicating significant differences in the data.

From Table IX it appears that the best order for this chromosome is: *dv* 3 *m* 2 *d* 4 *p* 10 *aw* (*ps o ro*) 13 *Me* (*Cu bk*) 4 *Wo* 2 $ms_{10}$ 5 *s*. The five genes in brackets appear to fit in the positions indicated but the recombination values

TABLE IX

Summary of the recombination values

| | *m* | *d* | *p* | *aw* | *ps* | *o* | *ro* | *Me* | *Cu* | *bk* | *Wo* | *ms*$_{10}$ | *s* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *dv* | 7<br>46 | 3 | 15 | 19 | 9 | 28 | 20 | — | 11 | 36 | 27 | — | 30 |
| *m* | | 2<br>19 | 5 | 15 | — | 28 | 11 | 25 | 25 | 20 | 30 | 36 | 25 |
| | *d* | | 4 | 14<br>24 | 12 | 15 | 27 | 23 | 27 | 25 | 32 | 27 | 30 |
| | | *p* | | 10 | 16 | 18 | — | 15 | — | 23 | 25 | — | 29 |
| | | | *aw* | | 40 | 40 | 18 | 13 | 8 | — | 26<br>16 | 30 | 35 |
| | | | | *ps* | | 26 | — | — | — | 22 | 13 | 15 | 20 |
| | | | | | *o* | | — | — | — | 18 | 21 | — | 23<br>34 |
| | | | | | | *ro* | | — | — | — | 26 | — | — |
| | | | | | | | *Me* | | — | — | 14<br>4 | — | — |
| | | | | | | | | *Cu* | | — | 1 | — | — |
| | | | | | | | | | *bk* | | 15 | — | 9 |
| | | | | | | | | | | *Wo* | 2 | | 7<br>19 |
| | | | | | | | | | | | | *ms*$_{10}$ | — |

between the genes within each bracket are not known. There is also evidence to indicate that *Wo*, and *aw*, have been involved in an inversion and are duplicated in certain crosses, but this evidence must be subjected to further critical tests. No new data for *ne* or *Lc* are presented here.

## Acknowledgment

I gratefully acknowledge the help given by the Dept. of Horticulture, Ontario Agricultural College, which furnished greenhouses, land, and other facilities for growing most of these plants.

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# CYTOLOGICAL STUDIES IN THE GENUS ERIGERON[1]

F. H. MONTGOMERY AND SHUNG-JUN YANG

## Abstract

The literature relating to the chromosome numbers in the genus *Erigeron* has been reviewed, and all reported numbers are given. The chromosome number for *E. breweri* var. *porphyreticus* (Jones) Cronquist, $2n = 18$; *E. divergens* var. *cinereus* A. Gray, $2n = 27$; *E. glabellus* var. *glabellus* Cronquist, $2n = 36$; *E. glabellus* var. *pubescens* (Hook.) Cronquist, $2n = 36$; *E. hyssopifolius* Michx., $2n = 18$; *E. quercifolia* Lam., $2n = 36$; *E. simplex* Greene, $2n = 27$; *E. strigosus* var. *beyrichii* (Fisch. & Mey.) A. Gray, $2n = 27$; and *E. vernus* T. & G., $2n = 18$ are new determinations. Different numbers from those previously reported have been found for *E. pulchellus* Michx., $2n = 27$, and *E. strigosus* (Muhl.) Willd., $2n = 36$. Chromosome counts over a large area of their distribution have been made for *E. annuus* (L.) Pers., *E. pulchellus* Michx., *E. philadelphicus* L., and *E. strigosus* (Muhl.) Willd.

## Introduction

Many genera of the tribe Astereae, family Compositae, are difficult taxonomic groups because clear-cut lines of differentiation between genera are not always evident. Also, species within the genera are highly variable and there are many reports of interspecific hybridization.

The genus *Erigeron* is no exception; it has its origin within the genus *Aster* and in turn has been considered by Cronquist (7) to be the parental stock for the genera *Conyza* and *Achaetogeron*. The genus *Haplopappus* is also closely related, being separated from *Erigeron* chiefly on the basis of the color of the rays.

The genus is a large one and Index Kewensis lists about 350 species (s. lat.). Cronquist (7) in his monograph describes 133 species (s. str.) occurring in North America north of Mexico. Vierhapper (40) monographed the Alpine species of Europe and near Asia. These are the only critical taxonomic studies of the genus.

There has been no systematic cytological study of the genus, and the greatest number of chromosome counts that have been made have been the incidental results of embryological studies (1, 2, 3, 5, 8, 12, 13, 28).

The present work was initiated as a cytological study of the four weedy species present in Ontario, *E. annuus*, *E. philadelphicus*, *E. strigosus*, and *E. canadensis* (*Conyza*).[2] Later it was expanded to include as many species as could be obtained, particularly from eastern North America, and to bring together the scattered literature.

## Materials and Methods

Seeds of *Erigeron* species were obtained from a number of botanic gardens and interested individuals, and plants were also collected by the senior author during his field trips in Ontario and United States. Root tips were pretreated

[1]Manuscript received January 21, 1960.
Contribution from the Department of Botany, Ontario Agricultural College, Guelph, Ontario.

[2]Cronquist (6) has transferred *E. canadensis* to the genus *Conyza*, therefore, it is not included in this paper.

## TABLE I

Chromosome numbers in the genus *Erigeron*

| Species | Chromosome number n | Chromosome number 2n | Authority | Source of material |
|---|---|---|---|---|
| *E. acre* L. | | 18 | Rutland (31) | British |
| | | 18 | Löve and Löve (21) | Scandinavia |
| | | 18 | Löve and Löve (21) | Scandinavia |
| | | 18 | Löve and Löve (23) | Iceland |
| | | 18 | Huziwara (16) | Japan |
| | | 18 | Huziwara (17) | Japan |
| *E. acre* var. *politum* (Fr.) Hyl. | 9 | | Holmgren (13) (as *E. politus*) | |
| | | 18 | Löve and Löve (22) | Scandinavia |
| *E. alpiniformis* Cronquist | | 18 | Löve and Löve (23) | Iceland |
| *E. alpinus* L. | | 18 | Sokolovskaia and Strelkova (35) | |
| | 9 | | Mattick (25) | |
| | | 18 | Huziwara (16) | Japan |
| | | 18 | Montgomery and Yang | Smith College Botanic Garden, Mass. |
| *E. annuus* (L.) Pers. | 13 | 26 | Tahara (37) | Japan |
| | | 26, 27 | Suzuki and Taguti (36) | Japan |
| | | 27 | Okabe (27) | Japan |
| | | 27 | Huziwara (15) | Japan |
| | | 27 | Huziwara (16) | Japan |
| | | 27 | Montgomery and Yang | Guelph, Puslinch, Huntsville, Pancake Bay, Ont.; Steeleville, Theodosia, Union, Missouri |
| *E. atticus* Vill. | | 18 | Favarger (11) | Switzerland |
| *E. aurantiacus* Agl. | 9 | | Harling (12) | |
| *E. aureus* Greene | 9 | | Harling (12) | |
| *E. boreale* (Vierh.) Simm. | | 18 | Böcher and Larsen (4) | Greenland |
| | | 18 | Löve (20) | Iceland |
| | | 18 | Löve and Löve (23) | Iceland |
| | | 18 | Huziwara (16) | Japan |
| | | 18 | Jörgensen, Sörensen, and Westergaard (18) | Greenland |
| *E. breweri* var. *porphyreticus* (Jones) Cronquist | | 18 | Montgomery and Yang | Rancho Santa Ana, Calif. |
| *E. compositus* Pursh. | | 63 | Böcher and Larsen (4) | Greenland |
| | | 54 | Böcher and Larsen (4) | Montreal Bot. Garden |
| | | 54 | Huziwara (14) | Japan |
| | | 54 | Huziwara (15) | Japan |
| | | 54 | Huziwara (16) | Japan |
| | | 54 | Montgomery and Yang | British Columbia |
| *E. divergens* var. *cinereus* A. Gray | | 27 | Montgomery and Yang | Bot. Garden, Wroclaw, Poland |
| *E. flagellaris* A. Gray | 18 | 36 | Huziwara (15) | Japan |
| | | 36 | Huziwara (16) | Japan |
| | | 27 | Montgomery and Yang | British Columbia |
| *E. frigidus* Boiss. | 9 | | Harling (12) | |
| *E. gaudinii* Briegg | | 18 | Favarger (11) | Switzerland |
| *E. glabellus* Nutt. | 9 | | Holmgren (13) | |
| | 9 | | Careno (5) | |
| | 9 | | Harling (12) | |
| *E. glabellus* var. *glabellus* Cronquist | | 36 | Montgomery and Yang | Woolsley Sask. (H. Hood) |
| *E. glabellus* var. *pubescens* (Hook.) Cronquist | | 36 | Montgomery and Yang | Woolsley Sask. (H. Hood) |
| *E. glabratus* Hop. & Horn. | 9 | | Tahara (38) (as *E. dubius* var. *glabratus*) | Japan |
| | | 18 | Sakai (32) | Japan |
| | 18 | | Matsuura and Suto (24) | Japan |
| | | 18 | Reese (30) (as *E. polymorphus*) | |
| *E. glabratus* var. *angustifolius* Tatewaki | | 18 | Sakai (32) | Japan |

TABLE I (*Concluded*)

Chromosome numbers in the genus *Erigeron* (*Concluded*)

| Species | Chromosome number | | Authority | Source of material |
|---|---|---|---|---|
| | *n* | 2*n* | | |
| *E. glaucus* Ker. | 9 | | Pagni (28) | Bot. Garden Firenzi, plants from Calif. |
| | | 18 | Montgomery and Yang | Gottingen Bot. Garden |
| | | 18 | Montgomery and Yang | Rancho Santa Ana, Calif. |
| | | 18 | Montgomery and Yang | Bot. Garden Basel, Switz. |
| *E. humilis* Grab. | 18 | | Holmgren (13) | |
| | | 36 | Flovik (10) | Iceland |
| | | 36 | Löve (20) | Iceland |
| | | 36 | Löve and Löve (23) | Iceland |
| | | 36 | Jörgensen, Sörensen, and Westergaard (18) | Greenland |
| *E. hyssopifolius* Michx. | | 18 | Montgomery and Yang | Port Arthur, Ont. (C. E. Garton) |
| *E. mucronatus* DC. | | 36 | Fagerlind (9) | Material from Coimbra Bot. Garden, Portugal |
| | | 32 | Battaglia (1) | Bot. Garden, Pisa |
| | | 36 | Larsen (19) | |
| | | 36 | Montgomery and Yang | Bot. Gard. Neuchatel, Switz. |
| | | 36 | Montgomery and Yang | Bot. Gard. Rouen, France |
| *E. philadelphicus* L. | | 18 | Huziwara (15) | Japan |
| | | 18 | Mulligan (26) | Ottawa, Ont. |
| | | 18 | Huziwara (16) | Japan |
| | | 18 | Montgomery and Yang | Rondeau, Turkey Point, Alliston, Kapuskasing, Ont. |
| *E. pulchellus* Michx. | | 18 | Harling (12) | |
| | | 18 | Montgomery and Yang | Galt, Grand Bend, Hamilton, Rondeau Park, Ont. Pennsylvania |
| | | 27 | Montgomery and Yang | Blacksburg, Va. |
| *E. quercifolia* Lam. | | 36 | Montgomery and Yang | Florida (Dr. L. O. Gaiser) |
| *E. simplex* Greene | | 27 | Montgomery and Yang | Royal Bot. Gard. Edinb. |
| *E. speciosus* var. *macranthus* (Nutt.) Cronquist | 13 | | Holmgren (13) (as *E. macranthus*) | |
| | | 18 | Bergman (2) | |
| *E. strigosus* (Muhl.) Willd. | | 27 | Holmgren (13) | |
| | | 27 | Bergman (3) | |
| | | 36 | Montgomery and Yang | Puslinch, Ontario |
| | | 27 | Montgomery and Yang | West Plains, Alton, Union Centreville, Poplar Bluffs, Missouri; Waterloo, Guelph, Huntsville, Ontario |
| *E. strigosus* var. *beyrichii* (Fisch. & Mey.) A. Gray. | | 27 | Montgomery and Yang | Union, Sullivan, St. Clair, Missouri |
| *E. trilobus* (Dec.) Boiss. | 9 | | Quezel (29) | N. Africa |
| *E. uniflorus* L. | | 18 | Sokolovskaia and Strelkova (34) | |
| | | 18 | Löve (20) | Iceland |
| | | 18 | Böcher and Larsen (4) | Greenland |
| | | 18 | Löve and Löve (23) | Iceland |
| | | 18 | Jörgensen, Sörensen, and Westergaard (18) | Greenland |
| *E. uniflorus* var. *eriocephalum* (J. Vahl.) Cronquist | 9 | | Holmgren (13) (as *E. eriocephalus*) | |
| | | 18 | Löve (20) | Iceland |
| | | 18 | Löve and Löve | Iceland |
| *E. vernus* T. & G. | | 18 | Montgomery and Yang | Florida (Dr. L. O. Gaiser) |

in a saturated solution of paradichlorobenzene for 45 minutes; fixed in Carnoy (3 parts of absolute alcohol to 1 part of glacial acetic acid) overnight, and hydrolyzed in *N* HCl at 60° C for 15 minutes; root tips were placed on a slide in a drop of acetocarmine and squashed. In later work aceto-orcein was found to be more satisfactory. Temporary slides were examined and chromosomes

counted from at least 10 cells of each plant. Photomicrographs were taken and the temporary slides made permanent. Voucher specimens have been placed in the herbarium of the Ontario Agricultural College.

## Results

Very early in the work it was found that chromosome counts from meiotic materials were not reliable, particularly when investigating triploid or apomictic species.

Table I lists the species for which chromosome numbers are known. It also gives the authority for the observation and, where it has been available, the original source of the materials.

## Discussion

Darlington and Wylie (8) and Tischler (39) have given Chiarugi as determining the chromosome number for *E. alpinum*. In checking Chiarugi's papers we have not been able to find that he reported original determinations, but rather those of other workers. We have, therefore, omitted any reference to Chiarugi's papers in our literature.

In 1915, Tahara (37) reported diploid counts of $2n = 26$ for *E. annuus*. Later, Suzuki and Taguti (36) examining "normal" plants (with rays) of the same species found 26 chromosomes in some and 27 in others, but "mutant" plants (rayless) were found to have 27 chromosomes. Okabe (27) reported 27 chromosomes for *E. annuus* from Japan. All of our plants from widely separated areas in Ontario and from the Ozark region of Missouri proved to have a chromosome number of $2n = 27$. Bergman (2, 3) and Holmgren (13) reported 27 chromosomes for *E. cfr. annuus* but it has been pointed out by Soderberg (33) that their material was actually *E. strigosus*.

Böcher and Larsen (4) found $2n = 63$ for *E. compositus* from Greenland, and $2n = 54$ for specimens received from the Montreal Botanic Garden. They concluded that two races of *E. compositus* exist. It will be interesting to have results of further studies of plants from the Greenland flora. Japanese material and our plants from British Columbia have shown a diploid count of 54 chromosomes.

*E. flagellaris* from Japan is reported by Huziwara (15, 16) as having 36 somatic chromosomes. Material received by the authors from British Columbia proved to have a $2n$ number of 27. *E. divergens* var. *cinereus* (Fig. 2) which closely resembles *E. flagellaris* (Fig. 3) is also triploid.

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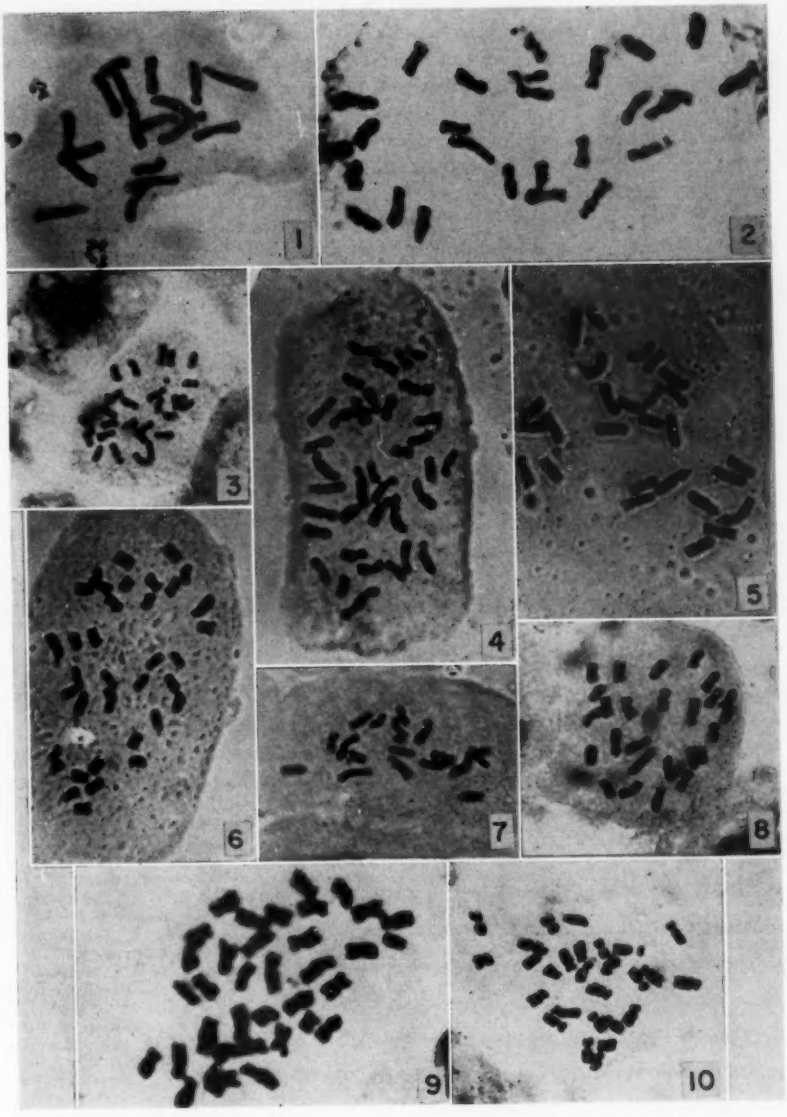
FIGS. 1–10. Somatic chromosomes of *Erigeron*. ×2100
FIG. 1. *E. breweri* var. *porphyreticus*, $2n = 18$.
FIG. 2. *E. divergens* var. *cinereus*, $2n = 27$.
FIG. 3. *E. flagellaris*, $2n = 27$.
FIG. 4. *E. glabellus* var. *glabellus*, $2n = 36$.
FIG. 5. *E. pulchellus*, $2n = 27$.
FIG. 6. *E. quercifolius*, $2n = 36$.
FIG. 7. *E. vernus*, $2n = 18$.
FIG. 8. *E. strigosus* var. *beyrichii*, $2n = 27$.
FIG. 9. *E. strigosus*, $2n = 36$.
FIG. 10. *E. simplex*, $2n = 27$.

PLATE I




Montgomery and Yang—Can. J. Botany

Our material of *E. glabellus* from Saskatchewan proved to include two varieties, var. *typicus* Cronquist (Fig. 4) and var. *pubescens* (Hook.) Cronquist. Both varieties were tetraploids, $2n = 36$. All other reports (15, 12, 13) have given the diploid number 18 for the species. Dr. Cronquist (in litt.) stated that in the area from which this material was received, Woolsley, these varieties overlap in their distribution.

Battaglia (1) gave $2n = 32$ for *E. mucronatus* from the Botanic Garden at Pisa, while plants from the Botanic Garden at Coimbra, Portugal, were reported by Fagerlind (9) to have a diploid number of 36. Battaglia concluded that two races of this species must exist.

Plants of *E. pulchellus* from widely separated areas in Ontario and in Pennsylvania had a chromosome number of $2n = 18$, but the senior author collected triploid material $2n = 27$ (Fig. 5) on route 460, about 5 miles north of Blacksburg, Virginia. These plants were growing on the gravelly road ballast along the edge of a woods. Further cytological studies in this area might determine whether both diploid and triploid races are present. A high degree of sterility occurs in both the diploid and triploid plants of this species. Counts by the authors have shown that as high as 85% of the florets lack embryos.

*E. strigosus* proved to have two chromosomal races. In an open woods in Puslinch Township near Guelph, specimens were found that were tetraploid, $2n = 36$ (Fig. 9). All our other collections from Ontario and from the Ozark Region of Missouri were triploid, $2n = 27$. *E. strigosus* var. *beyrichii* from the Northern Ozark region was also triploid (Fig. 8).

## Acknowledgments

This study was assisted in part by grants from the Canada Department of Agriculture, Science Service, and from the Ontario Research Foundation, and we express our appreciation for their generosity. Dr. Arthur Cronquist very kindly aided us in the identification of some species.

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# MEASUREMENT OF OXIDATION-REDUCTION CONDITIONS IN WHEAT LEAF SAP BY A POTENTIOMETRIC METHOD[1]

RUDOLF KAUL[2] AND MICHAEL SHAW

## Abstract

Extracts from primary leaves of Little Club wheat, prepared under anaerobic conditions, gave oxidation–reduction potentials in the range of −180 mv to −140 mv at pH 6 when measured potentiometrically with prepolarized platinum electrodes. These poorly poised potentials are believed to represent a flavin system. By mixing mediators (e.g. methylene blue and riboflavin) with the extracts stable average potentials were achieved. These ranged from −70 to +70 mv. Coleoptiles and very young primary leaves exhibited relatively low average potentials, which climbed to a steady level in 8 to 10 days after sowing. This change was accompanied by a quantitative shift between three poising systems found in the leaves by redox titrations. The results suggested that ascorbic acid becomes the dominating redox system in extracts from mature leaves. At senescence, indicated by yellowing, a new system dominates the redox background. This exhibited quinonic properties.

## Introduction

There are wide differences in the concentrations of the oxidation–reduction systems in plant tissue. Systems which occur in relatively high concentrations, e.g. ascorbic acid/dehydroascorbic acid and phenols/quinones, determine the redox background or "poise" of the tissue. Other systems like the cytochromes, flavins, or pyridine nucleotides occur in much lower concentrations. In higher plants the differences in concentration are so great that equilibration between the various systems may be expected to affect the ratio of oxidized to reduced forms of the 'poising' systems only to a minor extent. Thus the function of the poising systems is not only to serve as an energy reservoir but also to protect or "buffer" the natural redox organization against any serious change.

Measurement of redox states in living cells is extremely difficult. The method involving the least disturbance of the tissue is the use of redox dyes. Nevertheless these alter the redox properties in living cells considerably, partly by their own electron capacity and catalytic action and partly by their toxic effects on metabolism. Thus more recent attempts to measure redox conditions in living cells have utilized potentiometric techniques (4, 11). These techniques necessarily involve complete disturbance of the natural redox organization by bringing an electrode into contact with the cell contents and the validity of the more or less stable potential established in this way is open to serious criticism. Several electrode characteristics, the presence of electrode 'poisons', lack of affinity between electrode and medium or between systems in the medium itself, precipitation on the electrode surface, and absorption of gases by the metal of the electrode may all lead to artifacts in

[1]Manuscript received January 11, 1960.
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the measurement of redox potentials. In addition, traces of air in the system may cause serious oxidative changes in the electrode potentials. Reliable measurements of the true redox potentials in a complex system such as plant sap are only possible if careful attention is given to such details.

## Apparatus and Methods

### (*a*) *General*

All the potentials listed in this paper were read originally from a platinum electrode against a standard reference electrode of the saturated calomel type, using a Beckman model G pH meter. This instrument has a current uptake small enough to eliminate polarization tensions. $E_h$ values with reference to the standard hydrogen electrode were obtained by subtracting the voltage difference between $Pt/H_2$ at 0° C and 760 mm Hg and the saturated calomel electrode at 0° C (i.e. 249 mv). A final correction was then made for small pH fluctuations. The electrodes were arranged in a special vessel which allowed readings to be taken with a small volume of solution. The temperature in the electrode vessel was not controlled; however, all final potential values were taken between 20 and 25° C in order to avoid any appreciable effects of temperature.

### (*b*) *The Electrode Vessel*

An electrode vessel must possess arrangements serving for attachment of the electrodes, gas inlet and outlet, introduction of the burette for titration, and installation of a stirrer. Ground joints proved to be convenient for these purposes. In Fig. 1 an arrangement is shown which worked satisfactorily even with poorly poised systems. The bubbler (B) maintained a slight positive pressure of nitrogen in order to prevent diffusion of atmospheric oxygen into the cell. It was not necessary to grease the joints. For very poorly poised systems a special bubbler, filled with mercury, held a positive pressure of 40 mm Hg. The gas outlet attached to the bubbler was connected by means of rubber tubing to the titration equipment, in which the nitrogen passed through compartment C, containing the titration agent, before entering the atmosphere.

The reference electrode (K) entered the vessel through joint *a*. A compact unit of the saturated calomel type was used. In order to reduce diffusion of potassium chloride to negligible amounts a fine asbestos fiber (*f*) was sealed into the tip of the electrode until a thin layer of glass covered the lower end of the fiber. Thereafter a micro hole was punched through the glass cover by evacuating the electrode container and applying a high electric tension to the tip. Electrodes prepared in this manner showed no appreciable loss of potassium chloride after nearly 2 years.

Bright platinum was used for making the noble metal electrode (P). A platinum disk about 2 mm in diameter was welded to platinum wire and the latter sealed into the soft glass end of a graded seal (Corning Glass Works). The pyrex end of the seal was connected with joint *b*. By fusing the platinum wire with a copper wire, contact with the upper jack of the electrode was established.

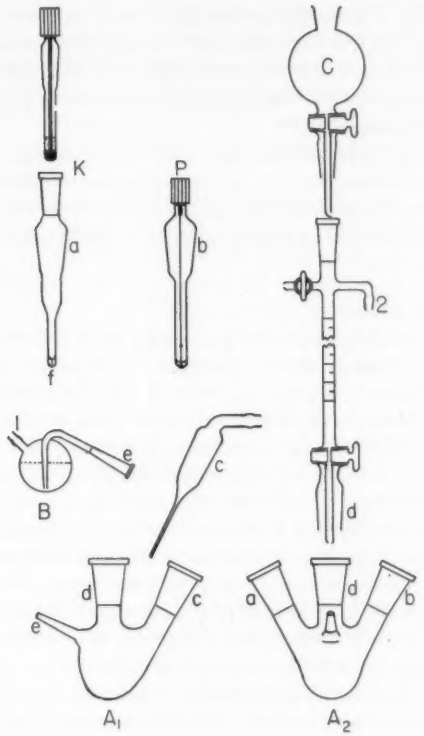


FIG. 1. Electrode set and vessel used for redox titrations. $A_1$ = electrode vessel, side view. $A_2$ = electrode vessel, front view. K = calomel electrode. P = platinum electrode. Points 1 and 2 were connected by means of rubber tubing in order to pass nitrogen through compartment C containing the titration agent. For further explanation see text.

*(c) Cleaning the Nitrogen*

In order to prevent interference with the redox equilibrium nitrogen is usually used to flush air out of the electrode vessel. Frequently, commercial nitrogen is purified by passing it over copper filings at 450° C. The equipment used is rather expensive and complicated. Furthermore, the low oxygen capacity per unit weight of copper filings requires large columns if frequent regeneration is to be avoided.

Schulek and Pungor (10) have reported the use of MnO for removing traces of oxygen. Following their technique MnO was prepared from $MnSO_4$ and $NaHCO_3$. The oxide was mixed with asbestos wool (weight ratio about 1:2) and a pyrex tube 1.0 in. in diameter and 16 in. long was filled with the mixture. The column was heated by a 750-w master element which was drawn out and wound around it. An autotransformer served as temperature regulator. Usually a temperature of 200° C was sufficient to remove traces of oxygen

from the nitrogen. The bright-green MnO was converted to black-brown $MnO_2$ and a sharp dark front indicated how far the column was exhausted. One column was sufficient to clean about 100 cubic feet of commercial nitrogen (0.5% $O_2$). Reduction of the column with hydrogen required a temperature of 400° C for 3–4 hours.

A trap containing layers of fine glass wool and charcoal was inserted ahead of the MnO train to absorb spray and other microparticles present in the $N_2$. No further equipment was necessary for purifying the nitrogen. All connections were made from Tygon plastic tubing in order to avoid diffusion of atmospheric oxygen into the system.

(*d*) *Pretreatment of Platinum Electrodes*

The insertion of a noble metal electrode into poorly poised systems, especially at low $E_0$ levels, requires several precautions in order to facilitate direct electron compensation between the systems and the electrode and to avoid quantitative alteration of the redox partners in the systems. Extremely thin surface layers of oxide form on platinum electrodes which are exposed for a long time to the air or stored in liquids into which oxygen diffuses freely. Formation of such layers will alter the electrode potential considerably. Another factor responsible for false readings is the electron capacity of the metal itself. The propagation of an electron tension from system to electrode involves a certain number of electrons so that the measurement itself results in evident potential shifts if the system is poorly poised. Finally, gaseous oxygen absorbed by the electrode may disturb the redox equilibrium in the sap or may cause extremely slow approaches to the final potential.

Polarization of the platinum electrodes was found to be an effective treatment against interference by the different phenomena mentioned above. Polarization was accomplished as follows: Two polyethylene containers were fused together in a flame and a hole punched through the connecting wall. This unit was cleaned thoroughly and filled with 0.01 *N* sulphuric acid. The redox electrode and another platinum electrode were inserted, one in each of the two fused containers. These served as anode and cathode respectively when the circuit was closed over a 6-v lead accumulator. Hydrogen developing on the redox electrode reduced the platinum surface, displaced absorbed oxygen, and supplied an electron precharge to the electrode.

The platinum electrodes were stored in distilled water only. Before and after each measurement including the pretreatment they were rinsed with ethanol and deionized water alternately.

(*e*) *Buffers*

The measurements described in this paper were made with sap expressed under anaerobic conditions from primary leaves of young wheat plants. Such extracts prepared as described below had pH values varying from 5.7 to 6.2. Attempts were made to control the pH of the extracts at pH 6.0 by the use of buffers showing good buffering capacities within this range. Phosphate buffer, recommended by Michaelis (8), gave a voluminous precipitate as

soon as leaf extract was added. Succinate–borate buffer proved to be satisfactory, although the tendency of borate to form complex compounds with molecules having an orthohydroxy configuration and the participation of succinate in a natural redox couple (succinate–fumarate) appeared to be disadvantageous. For comparison a buffer containing acid phthalate and sodium hydroxide was tested. No turbidity was observed after mixing this buffer with leaf extract.

The two buffers were prepared in the following manner:

(1) Borate–succinate buffer: 4.77 g $Na_2B_4O_7.10H_2O$ + 1.425 g succinic acid were dissolved in 100 ml water. Slight deviations from pH 6.0 were corrected by adding small amounts of 0.1 *M* borate or 0.1 *M* succinic acid respectively.

(2) Acid potassium phthalate – sodium hydroxide buffer: 0.2 *M* potassium biphthalate and 0.2 *M* sodium hydroxide solutions were mixed in the proportion 54.55/45.45 to give pH 6.0.

Usually 0.5 ml buffer solution was pipetted into the vessel. In order to read potentials at low levels the buffer alone was mixed with the plant extract. In later experiments traces of riboflavin and thionine or methylene blue were added in order to mediate systems at higher $E_h$ levels to the electrodes. Simultaneously the mediators were supposed to equilibrate electromotively sluggish systems in the extracts to one average potential.

Our original doubts concerning the use of succinate–borate buffer proved to be unfounded. The potentials listed in Table II were mediated far above the range of succinate–fumarate ($E'_0$ pH 7 −26 mv) by means of riboflavin – methylene blue. In tests employing different buffers no general change was noted. Moreover the titration curves shown in Figs. 4 and 5 showed no trace of the succinate–fumarate system. We assume that lack of succinic acid dehydrogenase in the extracts prevented electron interchange between the system (succinate–fumarate) and any hydrogen acceptor (methylene blue), the latter forming a link to the electrode.

(*f*) *Preparation of the Electrode Vessel*

All attachments except the platinum electrode were inserted into the vessel, and nitrogen was blown through at a slow rate (five bubbles per second). Next the buffer solution and a microdrop of *n*-octanol were added and the polarized platinum electrode was inserted. Carefully shielded copper cable connected the Pt-electrode to the valve voltmeter (Beckman model G). Cable shielding, the electrode support, and the autotransformer (for heating the nitrogen cleaning equipment) were 'earthed' with special cables. Finally, the bubbler was lifted and the leaf extract injected into the vessel.

The vessel and burette shown in Fig. 1 were employed for redox titrations. A simpler vessel without a titration attachment served for ordinary redox measurements.

(*g*) *Anaerobic Preparation of Extracts*

A small plug of pyrex glass wool was fitted into a B-D-Champion veterinary syringe (10 cc). The freshly collected leaves were then added and the syringe

closed with a threaded cap which held the piston. The piston was loosened by turning the handle counterclockwise and nitrogen at 20 lb/sq. in. was blown through the lock adapter at the lower end of the syringe for at least 1 minute. Finally, the lock adapter was closed with a sleeve cap and the piston tightened by turning the handle clockwise. The tissue was disintegrated by freezing the whole syringe for 15 minutes in a Dewar beaker containing methanol – dry ice (−80° C). The syringe and its contents were thawed for 1 minute in a boiling water bath and the extract was injected into the prepared electrode vessel. About 1–2 ml extract served for ordinary $E_h$ determinations, 2–3 ml for redox titrations.

## Results and Discussion

*Systems at Lower Redox Levels*

Among all redox couples known to exist in living cells, those with high poising action can be distinguished from those with poor poise. Two characteristics are important for the poise of a system: (*a*) the concentrations of both redox partners; and (*b*) the ratio of oxidized to the reduced form. The higher the total concentration of the partners and the closer the molar ratio of the couple to 1, the better the poise or "redox-buffering capacity" of the system.

Higher plants in general contain two extremely poorly poised systems at low level: the flavins and the coenzymes. All attempts to read normal potentials of both coenzyme I (DPN) and II (TPN) potentiometrically which have been reported have failed, because of the electromotive inertia between the coenzymes and the electrode. Burton and Wilson (3) estimated equilibrium constants for the oxidation of (i) ethanol to acetaldehyde and (ii) isopropanol to acetone. Both of these reactions require alcohol dehydrogenase and DPN as hydrogen acceptor. The standard free energy change for the reduction of DPN allowed the calculation of $E'_0$ (pH 7; 25° C) DPN/DPNH. Values of about −320 mv were obtained with both reaction systems. The value for TPN, calculated by means of the glutamic dehydrogenase system, was −324 mv under the same conditions.

With the flavins direct potentiometric measurements have been found to be feasible (2, 5, 6, 7, 8, 12). Considerable changes of the half step potentials occurred when the free flavins were combined with their specific proteins. The normal potential ($E'_0$ pH 7) of free flavins varied from −220 to −200 mv whereas the flavoproteins gave about −130 to −50 mv.

Considering the flavoproteins as the respiratory catalysts with the lowest measurable range of redox potentials, wheat leaf extracts and platinum electrodes were expected to show electron interchange resulting in potentials within this range. This expectation assumes that simultaneous equilibration between the flavoproteins and systems with higher $E_0$, thus yielding the average potential or a steady drift towards the average potential, did not occur at a velocity sufficient to interfere with the measurement of equilibration between the flavoproteins and the electrode. As shown in Fig. 2 measurements

at a relatively low level were practicable. The electrode was precharged, so that the approach to the final potential was made from minus to plus. All attempts to approach the final potential from the positive side failed, giving final values around $E_h$ (pH 6) = 0 and extraordinarily wide differences between replicates. It must be stressed, however, that the final potentials shown in Fig. 2 and listed in Table I were not constant for longer than 10 minutes and ultimately developed a steady but small drift (1–2 mv in 10 minutes) which lasted from 10 to 20 hours until average potentials in the range of +20 to +70 mv were reached (see next section).

Electron tension compensation between electrode and extract followed a function of natural decay (Fig. 2), the factor which decayed being the overcharge of the electrode. The curves were polynomials of degree $n$, but a satisfactory fit to the experimental data was obtained using the first two terms of the progression (Fig. 2).

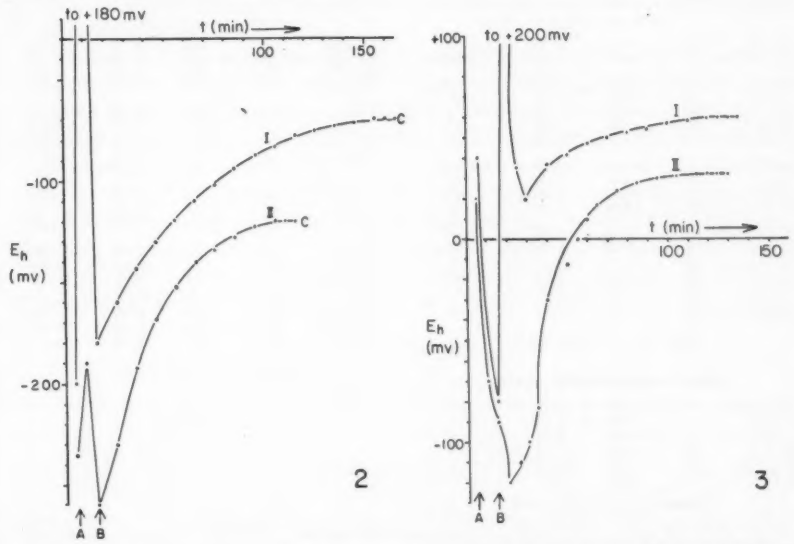


FIG. 2. Electron compensation between extracts from primary leaves of Khapli wheat and Pt-electrodes. At A, extracts were injected into prepared vessel; the electrodes at this point show the electron precharge obtained by polarization in diluted $H_2SO_4$. The logarithmic part of the curve followed the equation

$$\Delta E_t = E_g.e^{-K_1t-K_2t^2-\ldots-K_nt^n}$$

$\Delta E_t$ = Potential rise between B and any point on the curve $t$ minutes after B.
$\Delta E_g$ = Total potential rise (between B and C).
Curve I: Khapli, 14 days old; measurement on Dec. 10, 1958.
Curve II: Khapli, 15 days old; measurement on Sept. 3, 1958.

FIG. 3. Approach to average potentials in extracts prepared from primary leaves of Little Club and Khapli wheats. At A, steady potentials of polarized Pt-electrodes in buffer, containing methylene blue and riboflavin at $1.25\times10^{-5}$ $M$ each. Section A–B represents photoreduction of methylene blue by way of riboflavin. Extract injected at B.
Curve I: Khapli, 9 days old; measurement on March 26, 1959.
Curve II: Khapli, 14 days old; measurement on April 14, 1959.

A series of $E_h$ values obtained as final potentials are listed in Table I. Assuming that a flavin or flavoprotein system was responsible for these potentials, they were corrected to pH 6.0 using a factor of 60 mv per pH unit (7). Extracts from Little Club wheat grown in soil gave very constant final potentials ($E_h$ (pH 6.0) $-150 \pm 1.9$ mv, see Table I). Extracts from plants grown in water culture gave significantly lower potentials ($E_h$ (pH 6.0) $-178 \pm 1.93$ mv, see Table I). Extracts from Khapli wheat gave more variable potentials, especially during the winter months when supplementary fluorescent lighting was employed ($E_h$ (pH 6.0) $-128.6 \pm 8.86$ mv, see Table I).

TABLE I

Oxidation–reduction potentials (at lower levels) of wheat leaf extracts

| Little Club | | | | | Khapli | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Date (1958) | Days after sowing | $E_h$ (mv) | pH | $E_h$* (mv) | Date (1958) | Days after sowing | $E_h$ (mv) | pH | $E_h$* (mv) |
| Plants grown in soil | | | | | Plants grown in soil | | | | |
| 21 VIII | 15 | −133 | 5.75 | −148 | 26 VIII | 13 | −134 | 5.75 | −149 |
| 21 VIII | 15 | −129 | 5.75 | −144 | 26 VIII | 13 | −139 | 5.75 | −154 |
| | | | | | 28 VIII | 15 | −136 | 5.75 | −151 |
| 18 IX | 10 | −155 | 5.75 | −170 | 28 VIII | 15 | −126 | 5.75 | −141 |
| 18 IX | 10 | −152 | 5.75 | −167 | | | | | |
| 24 IX | 16 | −139 | 5.80 | −151 | 3 IX | 15 | −107 | 5.75 | −122 |
| 25 IX | 17 | −142 | 6.00 | −142 | 3 IX | 15 | −121 | 5.75 | −136 |
| 27 IX | 19 | −147 | 6.00 | −147 | 4 IX | 16 | −149 | 5.75 | −164 |
| | | | | | 4 IX | 16 | −146 | 5.75 | −161 |
| 1 X | 9 | −141 | 6.00 | −141 | 5 IX | 17 | −161 | 5.75 | −176 |
| 6 X | 14 | −149 | 6.00 | −149 | | | | | |
| 8 X | 16 | −150 | 6.00 | −150 | 1 XI | 15 | −121 | 6.00 | −121 |
| 11 X | 19 | −148 | 6.00 | −148 | 4 XI | 18 | −161 | 6.00 | −161 |
| | | | | | 6 XI | 20 | −129 | 6.00 | −129 |
| 13 XI | 7 | −155 | 6.00 | −155 | | | | | |
| 13 XI | 7 | −141 | 5.80 | −153 | 2 XII† | 13 | −131 | 5.80 | −142 |
| 18 XI | 12 | −126 | 5.60 | −150 | 2 XII† | 13 | −126 | 5.85 | −135 |
| 18 XI | 12 | −121 | 5.60 | −145 | 4 XII† | 15 | − 51 | 5.70 | − 69 |
| 24 XI | 18 | −126 | 5.60 | −150 | 5 XII† | 16 | 0 | 5.80 | − 12 |
| | | | | | 8 XII† | 12 | − 69 | 5.60 | − 93 |
| 28 XI | 9 | −132 | 5.80 | −144 | 10 XII† | 14 | − 71 | 5.50 | −101 |
| | | | | | 13 XII† | 17 | − 91 | 5.40 | −127 |
| Mean $E_h$ = $-150.24 \pm 1.9$ | | | | | Mean $E_h$ = $-128.63 \pm 8.86$ | | | | |
| Plants grown in nutrient solution | | | | | | | | | |
| 25 VIII | 9 | −166 | 5.75 | −181 | | | | | |
| 25 VIII | 9 | −161 | 5.75 | −176 | | | | | |
| 25 VIII | 9 | −161 | 5.75 | −176 | | | | | |
| 25 VIII | 9 | −161 | 5.75 | −176 | | | | | |
| 27 VIII | 11 | −160 | 5.75 | −176 | | | | | |
| 27 VIII | 11 | −171 | 5.75 | −186 | | | | | |
| Mean $E_h$ = $-178.33 \pm 1.93$ | | | | | | | | | |

NOTE: Difference between $E_h$* for soil- and solution-grown Little Club = 28.09 (significant at $P < 0.05$). $E_h$*= $E_h$ corrected to pH 6.0 by a factor of 60 mv/pH unit.
†Fluorescent lights at approximately 1000 ft-c from 5 a.m. to 11 p.m. plus daylight.

*Average Potentials*

By the use of redox mediators, it was possible to obtain potentials which were stable for more than an hour. The mediators were thought to catalyze equilibration between all redox systems in the extract and to facilitate electron interchange between electromotively sluggish systems and the electrode. Methylene blue ($E'_0$ (pH 7) +11 mv) and thionine ($E'_0$ (pH 7) +63 mv) were found to mediate poising systems in the extracts so satisfactorily that it was possible to make titration curves (Fig. 4). Riboflavin was added in order to make certain that all systems at lower redox levels would participate in the

establishment of average potentials. The use of either methylene blue or thionine together with riboflavin gave the same average potential for any particular extract. Methylene blue was used routinely. Usually 0.5 ml buffer, containing methylene blue and riboflavin at molal concentrations of $2.5 \times 10^{-5}$ each, was placed in the electrode vessel. Although these extremely small concentrations were not expected to oxidize the extract to a measurable degree, methylene blue was reduced via riboflavin by illuminating the vessel before injecting the extract (Fig. 3).

A list of average potentials is given in Table II. In general values obtained from plants which were harvested on sunny days were lower than those obtained under cloudy weather conditions. Very young leaves gave extremely low potentials, sometimes over 100 mv lower than those obtained from the same batch of plants 2 days later. This observation was made repeatedly and is demonstrated by the examples listed in Table II. Evidently the poising systems in wheat coleoptiles or in very young first leaves of wheat are different from those in older ones.

This assumption is supported by consecutive titration curves taken on extracts of the same plants with a 3-day interval (Fig. 4). The first two steps of curve *a* (extracts from leaves 8 days old) covered more than 50% of the oxidation range, whereas the third step amounted to about 40%. This picture was reversed 3 days later (curve *b*). The concentration of step 2 had decreased considerably. When growth of the primary leaves had stopped completely, step 3 covered nearly all the oxidation range (see Fig. 5*a*) and thus represents the only poising system in the cells. Possibly step No. 3, exhibiting an $E'_0$ value of +60 mv at pH 6, represents the ascorbic acid system. Ball (1) reported higher potentials for the ascorbic acid system under the same conditions

TABLE II

'Average' oxidation–reduction potentials of wheat leaf extracts

| Little Club | | | | | | Khapli | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Date (1959) | Days after sowing | $E_h$ (mv) | pH | $E_h$* (mv) | Weather conditions | Date (1959) | Days after sowing | $E_h$ (mv) | pH | $E_h$* (mv) | Weather conditions |
| 10 III | 6 | −66 | 5.80 | −72 | S | 4 III | 15 | +90 | 5.55 | +76 | C |
| 13 III | 9 | +32 | 5.80 | +26 | S | 4 III | 15 | +95 | 5.50 | +80 | C |
| 16 III | 12 | +31 | 6.10 | +34 | C | | | | | | |
| 17 III | 13 | +36 | 5.95 | +34 | S | 20 III | 9 | +51 | 5.90 | +48 | S |
| 18 III | 14 | +38 | 6.00 | +38 | C | 23 III | 11 | +53 | 5.90 | +50 | S |
| 20 III | 16 | +38 | 5.80 | +32 | S | 25 III | 13 | +64 | 5.90 | +61 | S |
| 2 IV | 8 | +69 | 5.90 | +66 | C | 23 III | 6 | +31 | 5.90 | +28† | S |
| 2 IV | 8 | +68 | 5.85 | +63 | C | 26 III | 9 | +59 | 5.90 | +56 | C |
| 3 IV | 9 | +71 | 5.90 | +68 | C | 29 III | 12 | +51 | 5.90 | +48 | S |
| 7 IV | 13 | +41 | 5.90 | +38 | S | 31 III | 14 | +59 | 5.90 | +56 | C |
| 10 IV | 16 | +49 | 5.90 | +46 | S | | | | | | |
| 13 IV | 19 | +46 | 5.90 | +43 | S | 25 IV | 10 | +41 | 5.90 | +38 | S |
| 17 IV | 23 | +52 | 5.80 | +46 | S | 27 IV | 12 | +40 | 5.90 | +37 | S |
| | | | | | | 30 IV | 15 | +39 | 5.90 | +36 | S |
| 7 IV | 7 | +29 | 5.90 | +26 | S | | | | | | |
| 11 IV | 11 | +29 | 6.00 | +29 | S | | | | | | |
| 14 IV | 14 | +31 | 5.90 | +28 | S | | | | | | |
| 22 IV | 22 | +48 | 5.85 | +43 | C | | | | | | |
| 3 VI | 8 | +29 | 6.0 | +29 | S | | | | | | |
| 6 VI | 11 | +40 | 6.0 | +40 | S | | | | | | |

NOTE: $E_h$* = $E_h$ corrected to pH 6.0 by a factor of 30 mv/pH unit. S = leaves harvested on sunny days; C = leaves harvested on cloudy days.

†Extracts red in color due to high anthocyanin content. Extracts were usually green.

($E'_0$ +88 mv at pH 6). His values were, however, corrected for a decrease caused by the instability of the oxidant at this pH. Considering the fact that the half-life time of dehydroascorbic acid at pH 6 is only 2 hours, a drop in the titrated $E_0$ values in our case must be taken in account (titration of the third step in Fig. 3 required 2–3 hours).

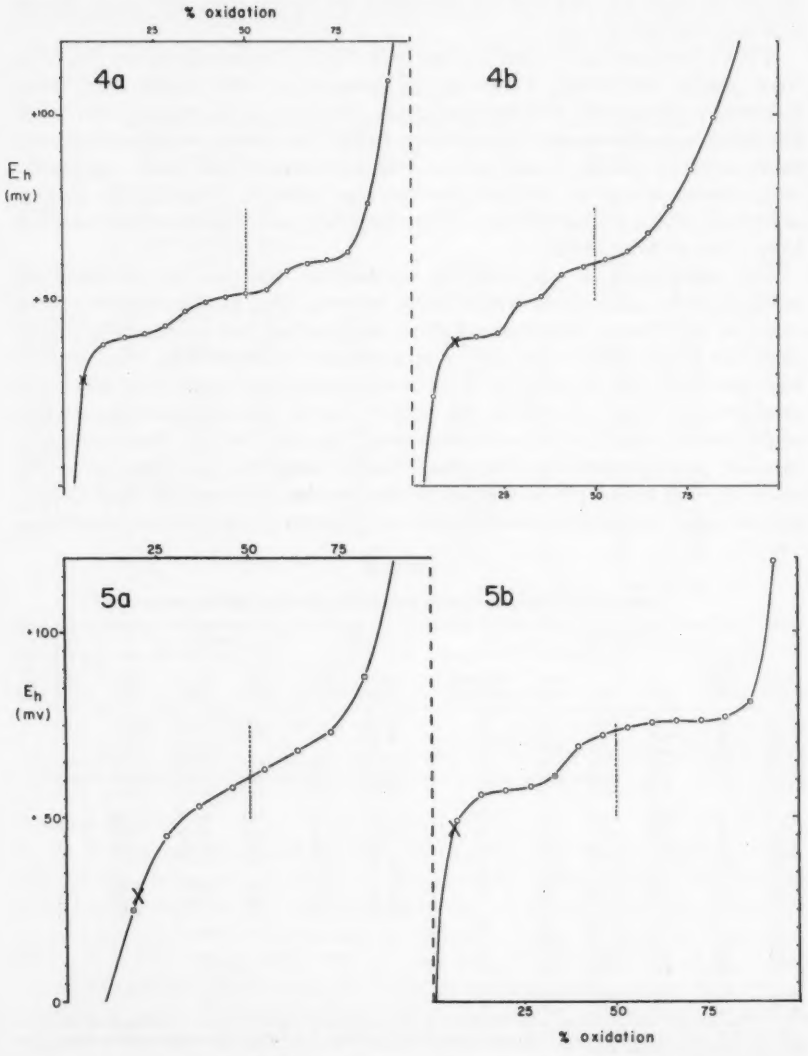

TABLE III

Empirical determination of pH correction factor employed in Table II

| Date (1959) | Days after sowing | pH A | $E_h$ A (mv) | pH* B | $E_h$ B (mv) | mv/pH | Wheat variety |
|---|---|---|---|---|---|---|---|
| 4 V | 13 | 5.90 | 49 | 5.00 | 78 | 32 | Khapli |
| 5 V | 8 | 6.00 | 49 | 5.10 | 84 | 39 | Little Club |
| 7 V | 10 | 6.10 | 59 | 5.00 | 89 | 27 | Little Club |
| 11 VI | 16 | 5.75 | 47 | 4.60 | 79 | 29 | Little Club |
| 12 VI | 10 | 5.85 | 50 | 5.15 | 73 | 32 | Little Club |
| | | | | | Average | 32 | |

*pH B adjusted by adding oxygen-free 0.2 *N* HCl dropwise to the extracts.

Figure 5, curve *b*, illustrates the titration of an extract from old, yellowing, primary leaves of Little Club. Surprisingly step No. 3, the ascorbic system, has decreased considerably in favor of a new, well-poised system at higher level ($E'_0$ (pH 6) about +75 mv). The formation of dark-brown melanine-like substances during the oxidative titration of step 4 suggests that the step represents a quinonic system.

In general, it can be said that the poising system in extracts of primary leaves of Little Club changes qualitatively and quantitatively during the aging process: the older the leaf, the higher the half step potential of the dominating system.

No explanation can be given for the changes shown in Figs. 4 and 5, especially as long as systems 1, 2, and 4 are unknown. The respiratory intensity depends on the systems which share in the chain of hydrogen transport. As Michaelis (9) pointed out, the concentrations of the participating systems and the rate of formation of free radicals as determined by enzymatic activation control the rate of oxidation reactions.

In Figs. 4 and 5 the original $E_h$ values of the extracts (marked ×) show that all systems were in the reduced state. Addition of 0.1 ml 1/100 molal $TiCl_3$ caused a potential drop of about 200 mv, thus indicating that practically no oxidized form was present.

The finding that normally three different systems were responsible for the redox poising in wheat leaf extract caused some difficulties with respect to the $E_h$ corrections to pH 6.0 (Table II). Provided the ascorbic acid couple is the

FIG. 4. Titration curves of extracts from young Little Club primary leaves. The extracts were injected into electrode vessels containing 1.0 ml borate–succinate buffer + methylene blue and riboflavin each at $2.5\times10^{-5}$ *M*. After average potentials (marked by ×) were reached 0.01 *M* $TiCl_3$ in 0.075% HCl was added. The addition of 0.1 ml gave $E_h$ (pH 6) −150 mv. Abscissae of Figs. *a* and *b* represent percentage oxidation achieved by titration with 0.01 *M* $K_4(Fe(CN)_6)$ dissolved in borate–succinate buffer (pH 6). The distance between two successive points on the curves represents 0.1 ml solution. Curve *a* illustrates titration of 2.4 ml of Little Club extract from plants sown May 26, 1959, harvested on June 3, 1959; curve *b* illustrates titration of 3.0 ml of extract from plants sown on May 26, 1959, and harvested on June 6, 1959.

FIG. 5. Titration curves of extracts from primary leaves of Little Club at mature (curve *a*) and senescent stages (curve *b*). Curve *a* illustrates titration of 2.3 ml of extract from Little Club plants sown on April 7, 1959, harvested on May 1, 1959; curve *b* illustrates titration of 1.9 ml of extract from Little Club plants sown on June 1, 1959, and harvested on June 25, 1959.

poising system, a correction factor of 30 mv/pH reported by Ball (1) is applicable. The two other unknown systems were closer to the original $E_h$ readings (× in Fig. 4) than ascorbic acid. Since their ionization contents were unknown it was not possible to determine the pH correction factor theoretically. Empirical determinations led to slopes varying around 30 mv/pH (Table III). This value was finally used to correct the values listed in Table II.

## Acknowledgments

The authors would like to thank Mr. William Hogg, Prairie Regional Laboratory, N.R.C., for his assistance with the glassblowing, and Mr. Ken Chaney for his help in the greenhouse. This work was carried out while one of us (R.K.) held a postdoctorate fellowship from the National Research Council for which grateful acknowledgment is made.

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# THE PHYSIOLOGY OF HOST–PARASITE RELATIONS

## VI. OXIDATION–REDUCTION CHANGES IN WHEAT LEAF SAP CAUSED BY RUST INFECTION[1]

RUDOLF KAUL[2] AND MICHAEL SHAW


### Abstract

Extracts of healthy and rusted leaves of Little Club and Khapli wheats were made under anaerobic conditions. Oxidation–reduction potentials in the extracts were measured at two different levels both in the presence and in the absence of both methylene blue and riboflavin as redox mediators. At the lower level (i.e. in the absence of mediators) a reversible rise in potential was noted during the course of rust infection on Little Club; with Khapli the rise was similar but was not reversed. The response to rust infection of resistant and susceptible plants was also reflected in characteristic changes in the average potentials at higher levels (i.e. in the presence of mediators). After infection the oxidation–reduction balance of susceptible tissue was reorganized by the introduction of new systems which were found in the reduced state only. After infection the oxidation–reduction potential of resistant tissue was only poorly poised as indicated by a rise in the average potentials and as demonstrated by oxidation–reduction titrations.

The results are discussed and the possibility is considered that a rust-induced catalyst may "short circuit" the chain of hydrogen transport, thus initiating all the observed redox changes.


### Introduction and Methods

A potentiometric method of measuring oxidation–reduction potentials in wheat leaf sap has been described (10). This method was employed to determine the effect of rust infection on redox conditions in resistant and susceptible varieties of wheat. Primary leaves of Little Club (rust reaction type 4), Khapli (rust reaction type 1), and Khapli I (rust reaction type 2-3) wheats were heavily inoculated with race 15B of stem rust (16). The leaf extracts used for redox measurements were prepared anaerobically at different stages of infection as described earlier (10).

In some instances, the extracts were also analyzed electrophoretically, using a Beckman-Spinco paper electrophoresis set. Twenty to 80$\mu\lambda$ of extract were applied to each paper strip. The buffer employed was a 0.1 *M* carbonate–bicarbonate mixture at pH 8.7 and was renewed after every second run.

### Results

(*a*) *Alterations in Potentials at Lower Levels* ($E_h$ *ca.* $-100$ *mv*) *During the Progress of Infection*

As described earlier (10), polarized platinum electrodes were used in making $E_h$ measurements at relatively low $E_h$ values, even though the potentials so measured were not stable for more than about 10 minutes. The $E_h$ values

[1]Manuscript received January 11, 1960.
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[2]National Research Council Postdoctoral Fellow.

obtained drifted in a characteristic manner as the rust fungus developed on Little Club (susceptible) and Khapli (resistant). With both varieties there was marked rise in $E_h$ during the "incubation" period, i.e. during the first 4–5 days after inoculation (Fig. 1). With Little Club this rise was subsequently reversed so that by the time the fungus sporulated freely the $E_h$ values were found to be very close to those for uninfected tissue. Death of the leaves led to a secondary rise in $E_h$ (Fig. 1). With Khapli there was an unreversed rise in $E_h$ after the incubation period (Fig. 1). It is noteworthy that the initial rise in $E_h$ accompanies the disease-induced rise in respiration. If we assume that a flavoprotein system produced the potentials in this $E_h$ range, then complete oxidation of this system must occur in rusted leaves of Khapli. An attempt was next made to determine whether systems at higher $E_h$ levels were involved by determining the average potential in the extracts.

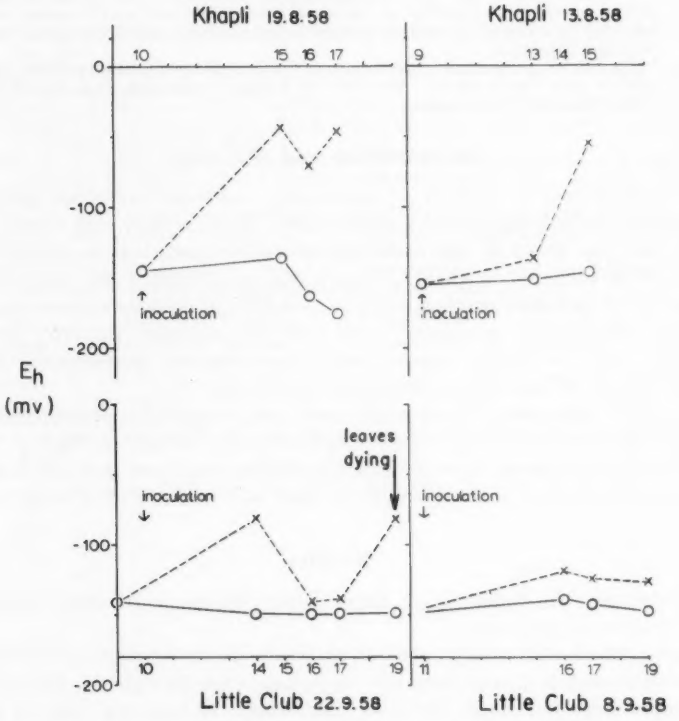


FIG. 1. $E_h$ values (pH 6) in wheat leaf extracts obtained without the use of mediators during different stages of rust infection. Abscissae: days after sowing. O———O healthy; X— — — —X rusted. Note the reversible potential rise in rusted Little Club extracts and the irreversible one in rusted Khapli.

(*b*) *The Drift in Average Potentials* ($E_h$ *ca.* $+50$ *mv*) *During the Course of Infection*

Average potentials were obtained by adding the redox mediators methylene blue ($5\times10^{-6}$ *M*) and riboflavin ($5\times10^{-6}$ *M*) to the extracts, plus potassium biphthalate – sodium hydroxide buffer at pH 6.0 (10).

The results obtained with Little Club and Khapli are summarized in Fig. 2. Khapli I, which is normally moderately susceptible, was completely resistant during the winter months. The heavily inoculated leaves turned yellow 3 to 4 days after inoculation and dried out within the next 2 days. The last samples were taken shortly before the leaves dried out. The extracts of these leaves were yellowish brown in color (extracts were normally green in color) and the average $E_h$ showed a clear rise in value. In the earlier stages of infection the $E_h$ values were virtually the same as for the controls. With Little Club the picture was just the reverse. Immediately after the infection flecks appeared the average potentials fell and reached a minimum at the stage of heavy sporulation (Fig. 2). These results suggested that a qualitatively new redox balance had been established in infected leaves of Little Club. A direct demonstration of such changes was achieved by redox titrations.

(*c*) *Titrations of Healthy and Rusted Leaf Extracts*

The curves in Fig. 3*b* represent the poising redox systems in healthy and heavily sporulating rusted leaves of Little Club. The extracts of healthy leaves appear to contain only one system ($E'_0$ (pH 6) = $+60$ mv) which is possibly the ascorbic acid system (10). Extracts of rusted leaves made 10

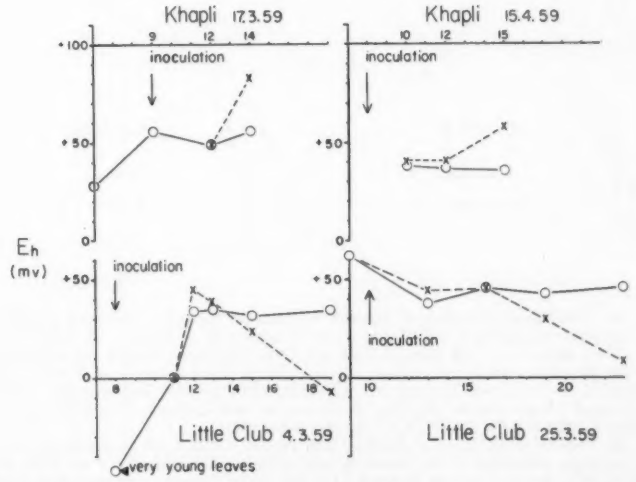


FIG. 2. Average potentials (pH 6) in wheat leaf extracts mediated by riboflavin and methylene blue in catalytic concentrations at different stages of infection. Abscissae: days after sowing. o————o healthy; x— — — —x rusted. Note the reverse reactions of Little Club and Khapli following rust infection.

days after inoculation contained two other systems, one at $E'_0$ (pH 6) = +50 mv, the other at $E'_0$ (pH 6) = +70 mv. The original average potential of the extracts, marked × in Fig. 3*b*, shows that all these systems were in the reduced form.

Since paper electrophoresis of these extracts revealed rather large amounts of chlorogenic acid, it is suggested that this acid represents one of the steps titrated in diseased Little Club extracts. Pure chlorogenic acid is, however, known to be electromotively inert with respect to platinum electrodes (15). We, therefore, tried to mediate between the electrode and the acid with different redox dyes (riboflavin, methylene blue, thionine, dichlorophenol–indophenol). These attempts were not successful. Wheat leaf extract was also added in an attempt to achieve enzymatic electron compensation between the electrode and chlorogenic acid. These attempts also failed. Nevertheless one of the primary oxidation products of chlorogenic acid may account

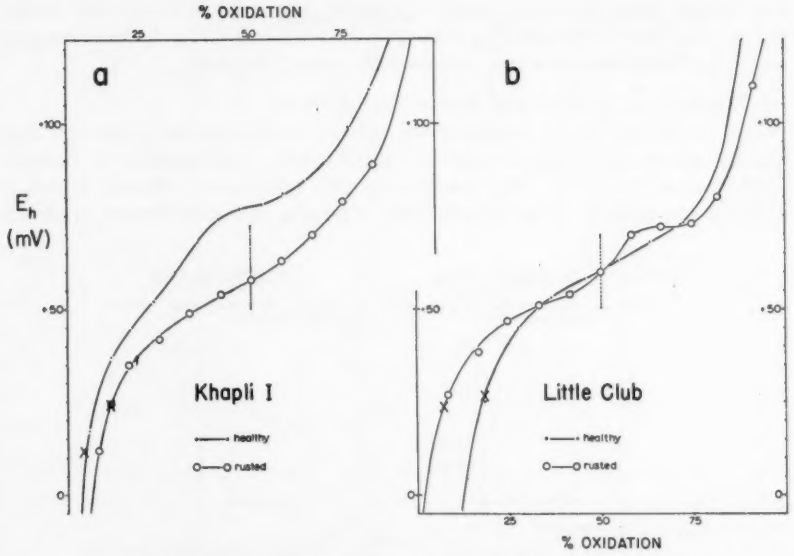


FIG. 3*a*. Oxidative titration of healthy and rusted Khapli I extracts at pH 6. The initial complete reduction of the extracts was achieved by adding 0.2 ml 0.01 *M* $TiCl_4$ in 0.075% HCl. ●——● Extract (2.1 ml) from healthy primary leaves titrated 14 days after planting. Standard oxidant consumption: 0.857 ml 0.01 *M* $K_3(Fe(CN)_6)$ per ml extract. O——O Extract (1.7 ml) from rusted primary leaves at the "yellowing" stage, titrated 14 days after planting (6 days after inoculation). Standard oxidant consumption: 0.706 ml 0.01 *M* $K_3(Fe(CN)_6)$ per ml extract.

FIG. 3*b*. Oxidative titration of healthy and rusted Little Club extracts at pH 6. The initial complete reduction of the extracts was achieved by adding 0.1 ml 0.01 *M* $TiCl_3$ in 0.075% HCl. ●——● Extract (2.3 ml) from healthy primary leaves titrated 20 days after planting. Standard oxidant consumption: 0.478 ml 0.01 *M* $K_3(Fe(CN)_6)$ per ml extract. O——O Extract (2.1 ml) from rusted primary leaves titrated 21 days after planting (10 days after inoculation). Standard oxidant consumption: 0.666 ml 0.01 *M* $K_3(Fe(CN)_6)$ per ml extract.

for one of the two steps found in extracts of rusted Little Club. Chlorogenic acid is known to yield a series of three oxidation products which show good interaction with platinum electrodes (15).

Healthy Khapli I leaves exhibited two systems which were not sharply separable by titration (Fig. 3*a*). The dominating system gave $E'_0$ (pH 6) close to +80 mv. The ascorbic acid system may well have been present, but may have been masked by the latter. Dark-brown polymerization products were formed during the oxidative titration and indicate the presence of phenolic compounds. In yellowing rusted leaves the dominating system was replaced by one with a half step potential close to +60 mv (pH 6.0). It is possible, but by no means certain, that this represents the ascorbic system. Again, these systems were originally present in the reduced state. These samples of Khapli I were grown in the late spring and were more susceptible to the rust fungus than those grown during the winter (see Fig. 2). Thus, after yellowing, the leaves remained alive for some days and developed rust pustules of type 2-3.

The titration curves for the resistant Khapli (type 1 reaction) are shown in Fig. 4. The important point to be noted is that, in contrast with all the data presented so far, the titration was a reductive one. The poising system in heavily infected resistant Khapli leaves is originally present in the extract

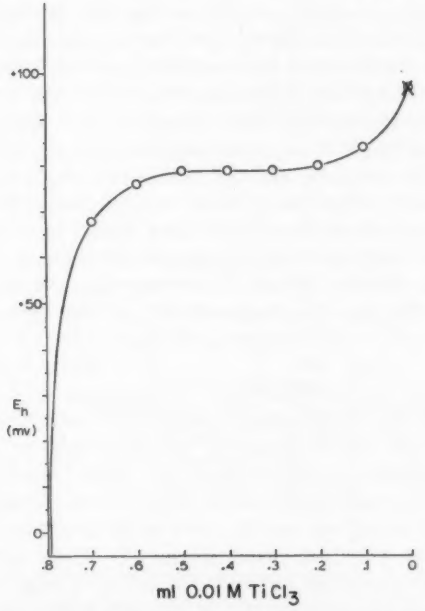


FIG. 4. Reductive titration (pH 6) of 1.8 ml extract from rusted primary leaves of Khapli titrated 24 days after planting (6 days after inoculation).

in the oxidized form only (marked by × in Fig. 4). Unfortunately, no data were obtained for healthy leaves of the same planting.

The foregoing shows that susceptible leaves rebuild their "redox background" and maintain it in the reduced state during the virulent stage of rust infection. This is, indeed, perhaps the most important attribute of "susceptibility". An irreversible rise in the average potential is a sign of resistance (see below). Khapli I was resistant during the winter months (see Fig. 2) and moderately susceptible during the summer, when light intensities were higher (Fig. 3*a*). In photosynthesis, light generates both reductive and oxidative power. Light also directly or indirectly stimulates certain other reductive and oxidative processes (nitrate reduction (3); oxidation of ascorbic acid (7) and indoleacetic acid (21)). These effects of light may be expected to interact at certain redox levels and it may be imagined that the balance between them determines the loss or gain of resistance. In "anaerobic" extracts and in vivo, all redox systems, especially the poising systems, tend to be in the reduced states. We suggest that any loss of electrons from these systems results in an increase in resistance.

A moderate increase in respiration usually occurs when the oxygen concentration is raised above that of air (9). It was expected that high oxygen tensions would produce relatively high average potentials in wheat leaves and would increase resistance to rust. Both expectations proved to be correct, although the oxygen-induced resistance was not permanent. Little Club plants in 6-in. pots were inoculated with rust in the usual way and kept on the greenhouse bench for 2 or 3 days. Several pots were then placed in a large, covered glass jar which was flushed continuously with a stream of oxygen containing about 0.1% (v/v) carbon dioxide. Under these conditions heavy inoculation did not result in any macroscopic symptoms of rust development. The average $E_h$ in similarly treated uninfected plants rose about 20 mv (Fig. 5). Those plants which remained on the greenhouse bench under normal conditions developed heavy infections. After 8 days of treatment, the inoculated plants were removed from the oxygen atmosphere and placed on the greenhouse bench. Within 3 days small rust pustules appeared, although the fungus never developed as well as it did on the untreated controls.

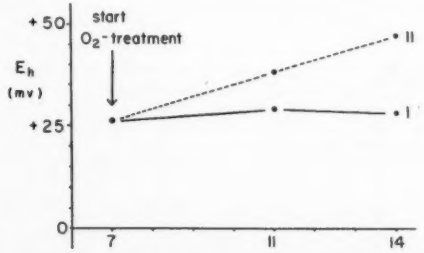


FIG. 5. Average potential changes induced by growing Little Club plants in oxygen + 0.1% $CO_2$; ●————● controls grown in air; ●— — — —● oxygen treated. Abscissa: days after planting.

## Discussion

The rapid increase in the respiration of the wheat leaf that follows rust infection is believed to be partly a response of the host to the presence of the parasite and has been attributed both to "uncoupling" of oxidative phosphorylation (13) and to increased synthetic activity at the infections (5, 17, 19). Of these two possibilities the available evidence favors the latter (1, 17, 19). There is a third possibility, namely that the increase in respiration is induced or partly induced by an oxidation–reduction catalyst. It is indeed well known that certain redox dyes (e.g. methylene blue and dichlorophenol–indophenol) cause large increases in carbon dioxide production and oxygen uptake (9). For example, Turner (see ref. 9) found that $1/10^4$ methylene blue raised the carbon dioxide output of well-washed carrot disks by 360%. The effect of a substantial number of phenolic compounds in stimulating respiration, even in intact plants and animals, may also be due to their redox function (2, 6, 12, 14). However, the work of Gaur and Beevers (8) suggests that the possibility that such compounds exert an "uncoupling" effect should not be overlooked.

The ability of exogenous redox catalysts to "short circuit" natural cell catalysts depends on (1) their affinity for certain enzymes, (2) their semiquinone formation constant at a pH corresponding to that maintained in vivo, and (3) their concentration.

Respiration would be increased if infection with rust resulted in the formation of a catalyst which participated in the chain of hydrogen transport and controlled and accelerated the speed of the over-all transfer of hydrogen to molecular oxygen. Such a catalyst could only maintain its controlling role if no other competing catalysts appeared. Thus it is tempting to speculate that an unidentified parasite-induced, redox catalyst may play a role in the acceleration of respiration at infections of obligate parasites. Such a mechanism of respiratory increase, involving qualitative and quantitative changes in the components of the hydrogen transport system, could also explain the shift which occurs in favor of the pentose phosphate pathway. It has in fact been shown that the respiratory increase caused by methylene blue involves a decline in the $C_6/C_1$ ratio, suggesting an increase in the participation of the pentose phosphate pathway (4).

No direct evidence in support of these speculations has been obtained in the present investigation and redox measurements and titrations of a more refined nature at several stages in the course of rust development are much to be desired. Nevertheless, the observed redox changes may have been initiated by a short circuit in the chain of hydrogen transport. The data that have been presented show that, during the aging of wheat leaves, as well as during the course of rust infection, relatively sudden changes occur in the redox systems that can be observed in leaf extracts. Infection first caused a rise in the redox potential of leaf extracts. In the susceptible variety, Little Club, this rise was subsequently reversed by the emergence of new poising systems in the reduced form. With the resistant Khapli the rise in

redox potential was not reversed and the loss of electrons led eventually to the formation of quinones and their dark-colored polymerization products, the former being detected by reductive titration. Accordingly it is postulated that resistance is associated with a rapid, unreversed oxidation of redox systems. Susceptibility implies the ability to reverse such oxidation or rather to maintain an adequate reducing potential. This concept of resistance and susceptibility is supported by the effect of oxygen in raising both resistance and the average potential of leaf extracts.

It has been found that rust infection of Little Club first stimulated and then strongly depressed indoleacetic acid "oxidase" activity as compared with uninfected controls (18). With the resistant variety, Khapli, the "oxidase" activity of infected tissue also increased and then fell off again, but remained well above that for uninfected tissue (18). It seems clear that these results for IAA-oxidase are broadly consistent with the drifts observed here in the redox potentials of wheat leaf extracts after rust infection. The "oxidase" results provide a specific example of the general trend now indicated by the redox measurements.

If indoleacetic acid is regarded as the reduced form of a redox pair, which is oxidized easily but reduced only with difficulty, then any other system with approximately the same $E_h$ value and with approximately equal affinity for factors which promote the oxidation of indoleacetic acid may be expected to protect the latter from such oxidation. Thus the harmonious interaction of several redox couples mediating between IAA and non-specific copper or iron oxidases probably regulates the rate of breakdown of IAA (11).

The problem of obligate parasitism may also be linked with redox potentials. In a recent publication Siebs (20) noted that germination of the conidia of powdery mildew of apple on artificial substrates was temporarily stimulated by roughly adjusting the redox status of the medium. It should not be forgotten, however, that the redox balance in living cells and tissues is a *dynamic* one, which would be difficult to reproduce in artificial media. A dynamic balance allows a stepwise arrangement of all redox pairs with respect to the redox scale, which is not disturbed by a certain oxygen tension. In a static system all the redox parts appear at the same average potential, which is maintained only by the careful exclusion of oxygen. If an obligate parasite requires specific redox conditions for its development, the medium itself will have to balance oxidative changes caused by the parasite. In turn it may be assumed that the host has to offer a steady energy flow from a certain redox level in order to feed the parasite. Perhaps only a system which maintains a steady state with respect to a dynamic redox balance can fulfill these requirements.

## Acknowledgments

One of us (R.K.) wishes to thank the National Research Council for the award of a postdoctoral fellowship. A grant (to M.S.) from the Canada Department of Agriculture is also gratefully acknowledged.

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# STUDIES IN SAXIFRAGACEAE

## III. SAXIFRAGA ODONTOLOMA AND LYALLII, AND NORTH AMERICAN SUBSPECIES OF S. PUNCTATA[1]

J. A. CALDER AND D. B. O. SAVILE


### Abstract

The taxonomy and phytogeography of *Saxifraga odontoloma* (*S. arguta* auct.), *S. lyallii*, and *S. punctata* are presented. *S. odontoloma* is not closely related to *S. punctata* but is much more closely allied to *S. lyallii* with which it hybridizes. *S. lyallii* is divided into two varieties, var. *lyallii* near the 49th parallel, and var. *hultenii* var. nov. from Alaska to southern B.C. *S. punctata* is divided into six subspecies in North America: sspp. *nelsoniana* (D. Don) Hult., *insularis* Hult., *pacifica* Hult., *carlottae* ssp. nov., *cascadensis* ssp. nov., and *porsildiana* ssp. nov.


### Introduction

Engler and Irmscher (3) divided *Saxifraga* sect. *Boraphila* Engler, with more than 60 species, into eight groups. Group 1, *Punctatae* Engl. & Irmsch., included 10 species, 6 of which are confined to eastern Asia; another species, *S. mertensiana*, which should be excluded from the group, will be discussed in a later paper. Of the remainder, the widely ranging *S. punctata* is clearly in need of revision and is discussed in this paper; *S. nudicaulis* is confined to the Bering Sea islands and eastern Siberia, and *S. spicata*, of Alaska and Yukon, seems to present no serious taxonomic problems. Group 2, *Davuricae* Engl. & Irmsch., included *S. davurica*, ranging from eastern Siberia to western Yukon, and *S. lyallii*. The latter, with two geographic populations, is discussed in this paper, together with *S. odontoloma* (*S. arguta* auct. not D. Don). The latter, treated by Engler and Irmscher and some other authors as a variety of *S. punctata*, is entirely distinct from it and must be transferred to the group *Davuricae*.

In floral morphology and pubescence *S. odontoloma* and *S. lyallii* are practically indistinguishable and, in fact, they hybridize rather freely wherever they meet. However, the zone of intergradation is abruptly delimited and relatively narrow, rather than forming a smooth cline, and it accordingly seems preferable to maintain the plants as separate species.

The study has been based on material from the herbaria listed below with their abbreviations:

CAN National Museum of Canada, Ottawa.
DAO Plant Research Institute, Dept. of Agriculture, Ottawa.
NY New York Botanical Garden.
S Naturhistoriska Riksmuseut, Stockholm.
UBC University of British Columbia, Vancouver, B.C.
US U.S. National Museum, Smithsonian Institution, Washington, D.C.
V Provincial Museum, Victoria, B.C.
WTU University of Washington, Seattle, Wash.

[1]Manuscript received November 26, 1959.
Contribution No. 40 from the Plant Research Institute, Research Branch, Canada Department of Agriculture, Ottawa, Ontario.

We wish to thank the curators of these institutions for the loan of material. We also wish to thank Dr. C. Frankton for making detailed notes on the isotype of *Saxifraga lyallii* at the Royal Botanic Gardens, Kew, and for assistance in Russian translations; Dr. B. Boivin for assistance with the Latin diagnoses; Dr. R. J. Moore for making chromosome counts; Mr. J. E. Dandy and Miss D. Hillcoat for providing detailed notes on the type of *S. arguta*; and Doctors H. S. Bostock and V. K. Prest for information on glaciology of the northern Cordillera.

## Taxonomy

Because of the frequent confusion of *Saxifraga odontoloma* with *S. punctata*, and the occurrence of hybrids between *S. odontoloma* and *S. lyallii*, it has seemed advisable to include these species in a single key with the subspecies of *S. punctata*.

1. Petals usually broadly elliptic to suborbicular with conspicuous claws and two yellow or green spots near base. (Spots sometimes absent in *S. lyallii* var. *lyallii*, or inconspicuous after drying in other taxa.)
   2. Plants generally 20.0–65.0 cm high; leaves orbicular and cordate . . . 1. *S. odontoloma*
   2. Plants 4.0–30.0 cm high; leaves spatulate to flabellate.
      3. Plants 4.0–14.0 cm high; leaves spatulate . . . . . 2*a*. *S. lyallii* var. *lyallii*
      3. Plants 9.0–30.0 cm high; leaves flabellate . . . . . 2*b*. *S. lyallii* var. *hultenii*
1. Petals narrowly to broadly elliptic, base cuneate or slightly clawed, unspotted.
   4. Panicle hairs mostly short, erect and prominently glandular; petals always white.
      5. Leaves glabrous to sparsely pubescent; panicle branches slender, panicle usually open especially in fruit; Siberian . . . . . 3*a*. *S. punctata* ssp. *punctata*
      5. Leaves definitely pubescent; panicle branches usually stout, panicle usually dense; essentially Alaskan . . . . . 3*b*. *S. punctata* ssp. *nelsoniana*
   4. Panicle hairs long, flexuous, eglandular or with small glands; petals white or tinged pink.
      6. Largest leaves with ca. 9–12 teeth; capsules 6.0–12.0 mm long . . . . . 3*c*. *S. punctata* ssp. *carlottae*
      6. Largest leaves with ca. 12–18 teeth; capsules 3.0–8.0 mm long.
         7. Panicle hairs appressed; plant slender; panicle open . . . . . 3*d*. *S. punctata* ssp. *cascadensis*
         7. Panicle hairs ascending; plants stouter; panicle often congested.
            8. Leaves thick and fleshy; capsules usually cleft 15–30%; Aleutians . . . . . 3*f*. *S. punctata* ssp. *insularis*
            8. Leaves thin; capsules cleft 25–75%.
               9. Largest leaves 2.3–7.7 cm wide, glabrous except slightly ciliate and a few hairs near petiole; capsules cleft 25–60%; Gulf of Alaska . . . . . 3*e*. *S. punctata* ssp. *pacifica*
               9. Largest leaves 1.0–3.8 cm wide, glabrous to sparsely pubescent; capsules cleft 40–75%; Yukon to Keewatin and northern Rockies . . . . . 3*g*. *S. punctata* ssp. *porsildiana*

1. SAXIFRAGA ODONTOLOMA Piper, Smiths. Misc. Coll. 50: 200. 1907.

   *Saxifraga odontophylla* Piper, Contr. U.S. Nat. Herb. 11: 314. 1906, *non* Wall. 1834.

   *Saxifraga arguta* auct., *non* D. Don.

   *Micranthes arguta* sensu Small, N. Am. Fl. 22, 2: 147. 1905.

   *Saxifraga punctata* L. var. *arguta* sensu Engl. & Irmsch., Pflanzenr. 4, 117 (67): 11. 1916, p.p.

   *Saxifraga punctata* L. ssp. *arguta* sensu Hult., Fl. Alaska and Yukon 5: 932. 1945.

Leaves generally orbicular, shallowly to deeply cordate, the largest 2.5–8.0(–10.0) cm wide, glabrous or occasionally with a few small hairs between the teeth or at the junction of the petiole and lamina, petioles up to 20.0 cm in length, glabrous; teeth to 1.3 cm long and to 1.3(–1.5) cm wide, triangular to mammiform, overlapping or open; scapes (10.0–)20.0–65.0(–75.0) cm high, glabrous below, becoming glandular above and in the inflorescence with pale to purple-black capitate glands; panicles (3.0–)7.5–32.0(–37.0) cm long, 2.2–13.0(–17.0) cm wide, branches upright or spreading, lightly to densely capitate-glandular; pedicels 0.3–0.5 mm in diameter; sepals (1.3–)1.8–2.8 mm long, 0.6–1.4(–1.8) mm wide, occasionally up to 3.3 mm long in fruit, green to purplish red, glabrous to occasionally minutely glandular-ciliate; petals 2.0–4.0(–5.0) mm long, (1.3–)1.7–3.7 mm wide, orbicular or broadly elliptic, always conspicuously clawed, with two yellow, green, or bicolored, occasionally confluent spots near base, white midvein obscure; filaments (1.2–)1.8–4.3 (–4.8) mm long, 0.2–0.7 mm wide, clavate or rarely elliptic, always slender at base, white to reddish; disk evident in flower, on purple capsules often evident as a green basal band sometimes with whitish dots; stigma 0.1–0.25 mm broad, barely distinguishable from beak; capsule (5.0–)6.0–9.0 mm long, 2.5–5.0 mm wide, cleft 30 to 60% of its length, beaks slightly to moderately divergent, varying from green to dark purplish red.

*Type.*—Slopes of Mt. Stuart, Cascade Mts., Washington, *Sandberg & Leiberg 570* (US) (mixed with *S. punctata* ssp. *cascadensis*).

*Range.*—Southern B.C., southward through Wash., Ida., Mont., Ore., Utah, Colo., Calif., Nev., to Ariz., and N.M. (Fig. 1).

Once it became clear to us that this taxon was specifically distinct from *Saxifraga punctata* we applied the epithet *arguta* to it, following the usage of Small (8) and other authors. The description by Don seemed applicable to this plant, but the type location was in doubt. Don stated that the type had been collected by Menzies. Menzies collected on the coasts of British Columbia and/or southern Alaska in 1787 and 1788 under Colnett, and in 1792–1794 under Vancouver. Only in 1792, when Vancouver charted Puget Sound and circumnavigated Vancouver Island, was Menzies in or close to the range of *S. odontoloma*. This part of his journal is available under the editorship of Newcombe (7). His account did not indicate that Menzies climbed any mountain likely to support this species. He records an unfamiliar *Saxifraga* from the cliffs of an island west of Bellingham, Wash., but this was perhaps *Lithophragma* sp. Belatedly, after returning many borrowed sheets of this plant annotated as *S. arguta* we appealed to Mr. J. E. Dandy for information concerning the type specimen of *S. arguta*. Information received from Mr. Dandy and Miss D. Hillcoat make it clear that Menzies' plant is *S. punctata*; the condition of the specimen renders subspecific identification somewhat uncertain, but it is apparently ssp. *pacifica* or intermediate between *pacifica* and *carlottae*. Accordingly it has been necessary to take up *S. odontoloma* Piper for the plant to which the epithet *arguta* has for so long been applied.

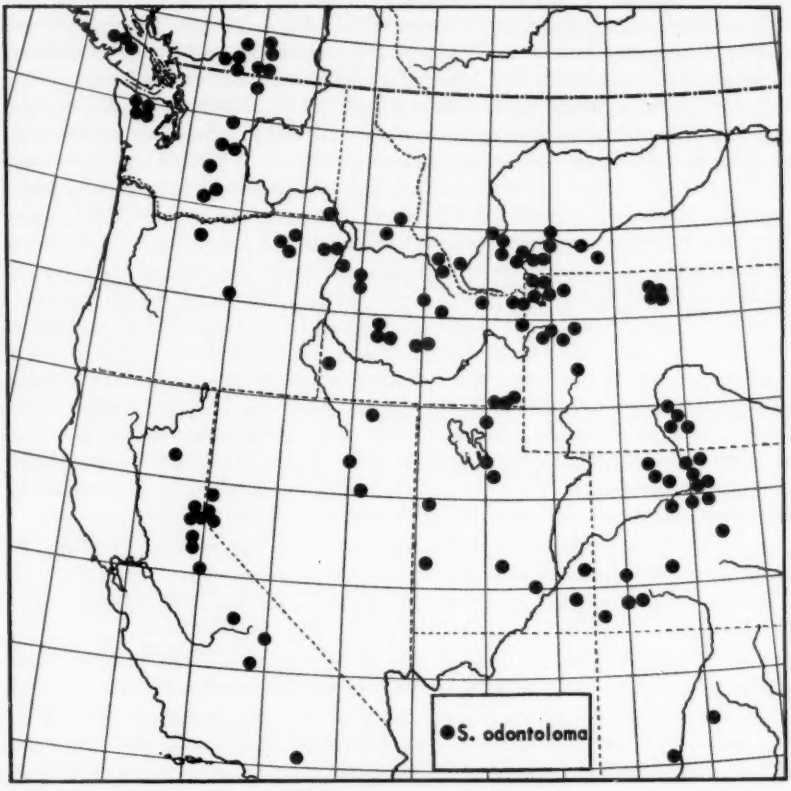


FIG. 1. Distribution of *Saxifraga odontoloma*.

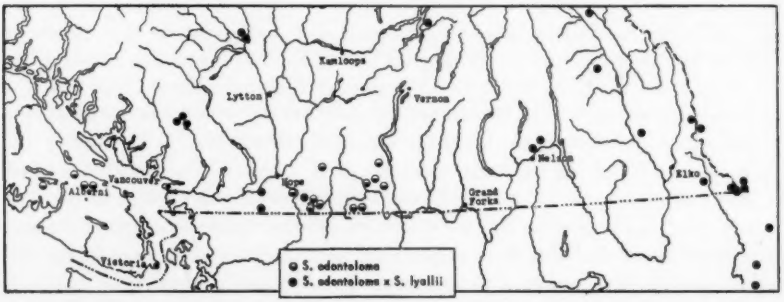


FIG. 2. Zone of junction between *Saxifraga odontoloma* and *S. lyallii*, showing occurrence of hybrids.

The synonymy of this plant has been further complicated by its confusion with *S. punctata* ssp. *cascadensis*. The type sheet of *S. odontoloma* bears a plant of each taxon, but Piper's description ". . . entirely glabrous up to the inflorescence . . . inflorescence glandular . . . petals orbicular and unguiculate" could not apply to *cascadensis*.

In Washington and southwestern British Columbia, where this species often grows with *S. punctata* ssp. *cascadensis*, it may be distinguished by its nearly orbicular, strongly clawed petals with two yellow or green spots and by the prominent, dark, short-stalked glands of the inflorescence. Despite their not infrequent close association no indication of hybridization between these plants has been found. In contrast, where *S. odontoloma* and *S. lyallii* meet, approximately along the 49th parallel, these species hybridize quite frequently (Fig. 2). These two species differ so markedly both in stature and in size and shape of leaves that the occurrence of hybrids between them was unexpected. However, *S. lyallii* has the distinctive petals and stalked glands of *S. odontoloma*, and it is clear that the species are actually very closely related. The hybrids are intermediate in stature, with leaves generally having a nearly orbicular blade but with the upper part of the petiole flabellate as in *S. lyallii*.

*Saxifraga odontoloma* occurs mainly from subalpine elevations downward along brooks and in other moist, shady situations. In general it is found at lower elevations than *S. lyallii*, but the two overlap where the latter comes down along alpine brooks.

Dr. R. J. Moore has determined the chromosome number to be $2n = 48$ for material from the Cathedral Lakes region, British Columbia. No count is available for *S. punctata* ssp. *cascadensis*, but the chromosome number of ssp. *porsildiana* is $2n =$ ca. 72, which provides indirect support for our contention that *odontoloma* and *cascadensis* do not hybridize.

Nearly all the available specimens are cited for the critical area near the northern limit of *S. odontoloma*, but the material from further south has been so abundant that only a small, representative selection is cited.

BRITISH COLUMBIA: Hope-Princeton Highway, Manning Park, *Calder & Savile 10607* (DAO); Allison Pass, Manning Park, July 27, 1945, *Hardy* (V); Quiniscoe Lake, Cathedral Lakes district, *Calder, Parmelee, & Taylor 19632* (DAO); Mt. Apex SW. of Penticton, *Calder & Savile 11693* (DAO); about 8 miles SW. of Okanagan Falls, *Calder & Savile 9927, 9930* (DAO); Mt. Brent west of Penticton, *Calder, Savile, & Ferguson 15774* (DAO); Trout Lake, Okanagan, *Wilson 485* (UBC); Sproat Lake, Vancouver Island, *Taylor 1243* (UBC); Mt. Arrowsmith, Vancouver Island, *Lohbrunner 9374* (V); Mt. Joan, Vancouver Island, July 15, 1943, *Wyatt* (V); second summit west of Skagit River, July 22, 1905, *J. M. Macoun* (CAN, NY); south of Tulameen River, July 27, 1888, *Dawson* (NY). MONTANA: Pintlar Falls, Beaverhead Co., *Hitchcock & Muhlick 12724* (NY); Red Lodge, Carbon Co., *Rose 12* (NY); Bozeman Canyon, Galatin Co., *Shear 3084* (NY); Basin, Jefferson Co., 1892, *Kelsey* (NY); Sheridan, Madison Co., 1895, *Fitch* (NY); Electric Peak, Park Co., *Rydberg & Bessey 4284* (NY); Boulder Creek, Stillwater Co.,

*Hitchcock & Muhlick 13376* (NY); Sweet Grass Canyon, Crazy Mts., Sweetgrass Co., *Flodman 516* (NY). IDAHO: Black Lake, Adams Co., *Christ 8626* (NY); 13 miles west of Bloomington, Bear Lake Co., *J. H. & C. B. Christ 18727* (NY); Devils Bedstead, Sawtooth Range, Blaine Co., *Thompson 13568* (NY); about 10 miles south of Lowman, Boise Co., *Hitchcock & Muhlick 9927* (NY); Spencer, Clark Co., *Christ 2881* (NY); near Mt. Borah, Lost River Mts., Custer Co., *Hitchcock & Muhlick 10929* (NY); Rocky Bar, Elmore Co., *Christ & Ward 8292* (NY); head of Cub River, Franklin Co., *Christ 16225* (NY); Sawtelle, Fremont Co., *Christ 5662* (NY); Seven Devil Mts., Idaho Co., *Christ 12688* (NY); 31 miles north of Challis, Lehmi Co., *Christ 15597* (NY); Silver City, Owyhee Co., *Macbride 439* (NY); Victor, Teton Co., *Christ 5294* (NY); near Cascade, Valley Co., *Thompson 13850* (NY). WASHINGTON: Blue Mts., Asotin Co., *Cronquist & Jones 5936* (DAO, CAN, NY); Mt. Stuart, Chelan Co., *Sandberg & Leiberg 570* (US, type in part); Olympic Mts., Clallam Co., *Elmer 2639* (NY); Olympic Mts., *Piper 2213* (NY); near Marmot Pass, Jefferson Co., *Thompson 9875* (NY); Beverly Creek, Kittitas Co., *Thompson 9587* (NY); below Big Craggy, Okanogan Co., *Thompson 10868* (NY); Mt. Rainier, Pierce Co., *Piper 2025* (NY); Mt. Aix, Yakima Co., *Thompson 15066* (CAN, NY).

Citations are omitted for the southern part of the range, in which no taxonomic problems exist.

S. LYALLII × S. ODONTOLOMA

ALBERTA: Sheep Mtn., Waterton Lakes National Park, *J. Macoun 10554* (CAN); Crowsnest Forest Reserve, Aug. 10–24, 1915, *Malte* (CAN); Cameron Lakes, Waterton Lakes National Park, *Taylor 9684* (UBC). BRITISH COLUMBIA: Alpine Mtn., near Nelson, *Calder & Savile 11105* (DAO); South Kootanie Pass, Aug. 6, 1881, *Dawson* (CAN); Kootanie Pass, Aug. 16, 1873–74, *Dawson* (CAN); Kokanee Provincial Park, July 29, 1938, *Eastham* (UBC); Garibaldi Provincial Park, Aug. 1913, *Davidson* (UBC), Aug. 12, 1917, *Davidson* (UBC); Paradise Valley, *Anderson 8284* (V); Timberline Valley, Manning Park, Aug. 3, 1945, *Hardy* (V); Mt. Cheam, *Anderson 6702* (V), Aug. 14, 1896, *Anderson* (V), Aug. 16, 1901, *Anderson* (V), Aug. 15, 1899 & 1901, *Fletcher & Anderson* (DAO); Queest Creek, *J. M. Macoun 8365* (CAN); Mt. McLean, Lillooet, *Calder, Savile, & Ferguson 15509A* (DAO), July 13, 1916, *J. M. Macoun* (CAN, V); Wall Lake, B.C.–Alberta border, *Taylor 8965* (UBC, in part), *Taylor, Calder, & Ferguson 3524* (DAO); Ice River south of Field, *Taylor & Ferguson 1889* (DAO); Lakit Mtn., 49° 43′ N. 115° 36′ W., *Taylor & Ferguson 2835* (DAO); Tornado Creek, 49° 59′ N. 114° 40′ W., *Taylor & Ferguson 3127* (DAO); Tamihi (Tami Hy) Mtn., Chilliwack Valley, Aug. 29, 1901, *J. M. Macoun* (CAN, in part); Flathead area, *Thomlinson 85* (DAO). MONTANA: Big Fork, Flathead Co., *Umbach 103* (NY, MONT); near Echo Lake, Swan Range, Flathead Co., *H. T. & J. M. Rogers 1278* (NY); Mt. Henry, Glacier National Park, *Umbach 575* (NY, MONT). IDAHO: Avery, Shoshone Co., *Christ 2462* (NY).

2*a*. SAXIFRAGA LYALLII Engler, Verhandl. zool.-botan. Ges. Wien, 19:542. 1869, var. LYALLII

*Micranthes lyallii* (Engler) Small, N. Am. Fl. 22, 2: 143. 1905.

Leaves spatulate, the largest 1.0–1.7 (3.0) cm wide, sides generally convex, with (3–)5–7(–11) triangular teeth often running down the sides, maximum length 2.5–4.5 cm including petiole, glabrous or at most slightly ciliate between the teeth. Scapes 4.0–15.0 cm high, glabrous below, with a few usually purple-black capitate glands above and on the panicle branches; panicles narrow 2.0–9.0 cm long, 0.6–2.8 cm wide, branches generally ascending, if spreading very short. Sepals about 1.5–2.6 mm long, green to purplish red, glabrous or occasionally minutely glandular; petals 2.0–3.5(–4.7) mm long, 1.2–1.8(–2.4) mm wide, elliptic to suborbicular, slightly to strongly clawed, unspotted or with two yellow or green spots near base, white or tinged pink; filaments 1.5–3.5 mm long, 0.1–0.4 mm wide, linear, elliptic, clavate, or more or less triangular, white or tinged pink; disk usually obscure in fruit; stigmas 0.1–0.25 mm wide; capsule 5.0–8.0 mm long (only a few seen), 3.0–4.0(–5.5) mm wide, cleft about 50% of its length, the beaks slightly to strongly divergent, varying from green to purplish red.

*Type.*—Rocky Mts., lat. 49° N. at 6500 ft elevation, 1861, D. Lyall (Isotype, K). See discussion below.

*Range.*—Southeastern B.C., southwestern Alta. and adjacent Mont.; and extreme southwestern B.C. and northwestern Wash. (Fig. 3).

The type locality of *Saxifraga lyallii* was given by Engler as near Fort Colville, Wash., a statement that has puzzled later botanists who have failed to find the species in that region. Although the Oregon Boundary Survey, to which David Lyall was attached as botanist and surgeon, spent the preceding winter of 1860–61 at Fort Colville, the party left in the spring too early for any subalpine plants to be in flower. As Lyall (6) showed in his journal, the party was in the lowlands during the early part of the summer. Not until they crossed the Macdonald Range in passing from the Kootenay to the Flathead Valley was any great elevation reached. It seemed clear, therefore, that the specimen must have been taken in either the Macdonald Range or the Rockies. The holotype in Berlin was presumably destroyed, but there is an isotype at the Royal Botanic Gardens, Kew. Dr. C. Frankton made notes and sketches of this specimen when visiting that institution, and his information clarifies the situation. The printed label heading runs: Herbarium of the Royal Gardens, Kew, OREGON BOUNDARY SURVEY, From Fort Colville to Rocky Mountains. The handwritten information runs: *Saxifraga Dahurica* Pall. Rocky Mts., Lat. 49° N. at 6500 ft. alt. Coll. by D. Lyall, 1861.

In his journal Lyall used the term "Rocky Mts." in its strict sense, excluding what is now called the Macdonald Range. It is therefore clear that his plant came from the Rockies, near the junction of British Columbia, Alberta, and Montana. Dr. Frankton's notes and sketches show the Kew isotype to be a small plant, 15.3 cm high, with a slender panicle and small, spatulate leaves. He could discern no petal spots. It is thus typical of the plants from this region.

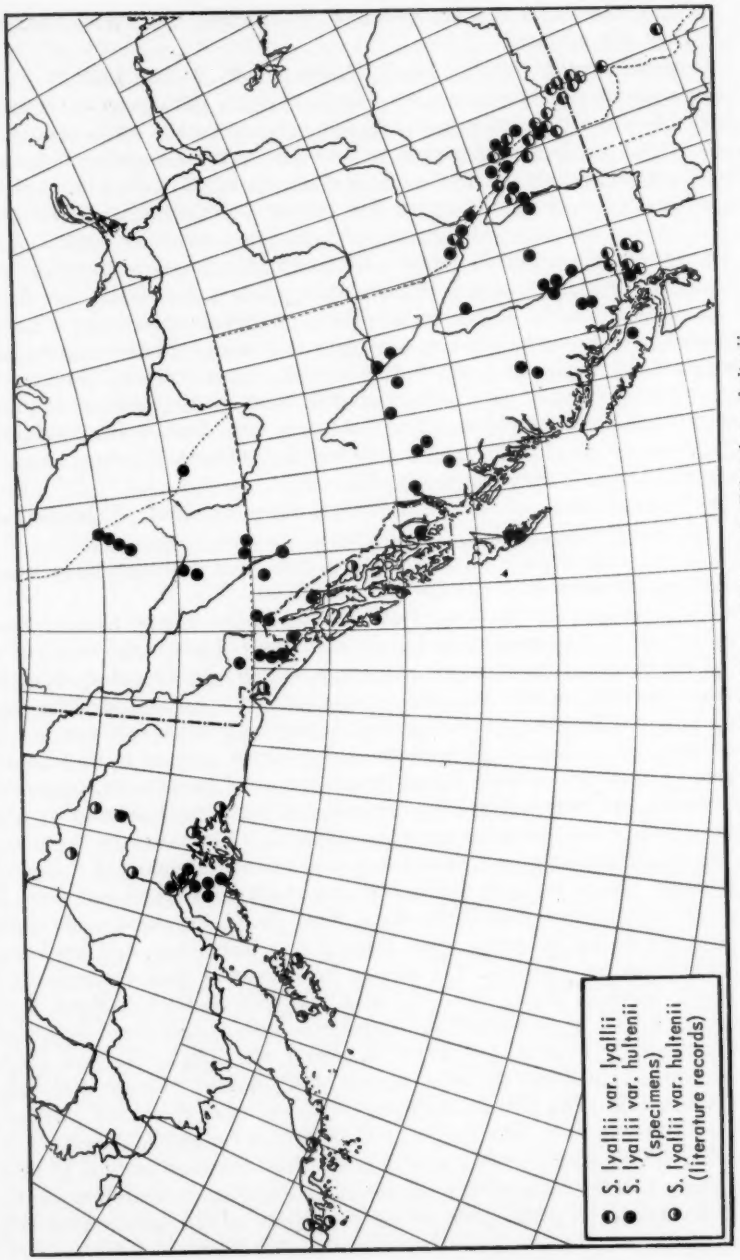


FIG. 3. Distribution of *Saxifraga lyallii* var. *lyallii* and var. *hultenii*.

Realization that *S. lyallii* consisted of two morphologically distinguishable populations came to us belatedly. It had been noted earlier that plants from southern Alaska and west-central British Columbia harbored the rust *Puccinia heucherae* var. *austroberingiana*, whereas those from southern British Columbia harbored *P. h.* var. *heucherae*. Dr. G. B. Cummins then sent us a specimen from northern Montana that proved to be *P. h.* var. *saxifragae* on a plant slightly different from most of those seen by us. This finding suggested the occurrence of a refugial population in Montana and prompted a thorough examination of all the available material. It was soon evident that the plants from the southeastern part of the total range were distinguishable, notably by their smaller and spatulate rather than flabellate leaves; and it was later found that similar plants occur sparingly in northwestern Washington and a narrow adjoining strip of British Columbia. The distribution pattern of this variety is somewhat obscured by the occurrence of the hybrids with *S. odontoloma* mentioned above. Because their ranges are not altogether distinct it seems preferable to treat the populations as varieties rather than subspecies. It is possible that var. *lyallii* is in process of extinction caught as it is between the larger and perhaps more aggressive var. *hultenii* and *S. odontoloma*.

Mention should be made here of *S. lyallii* var. *laxa* Engl. (Mon. Gatt. Sax. 172. 1872). Engler described var *laxa* from another Lyall specimen of 1861 "bei Fort Colville", although he later referred to this variety a collection from the Lynn Canal in the Alaska panhandle, which is presumably our var. *hultenii*. Var. *laxa* was stated to be larger, with taller flowering stems, larger basal leaves with blades to 4 cm wide, and petioles with the apex cuneate. The type of var. *laxa* presumably came from near the type locality of var. *lyallii*. Our evidence is that var. *hultenii* does not reach that region, and we suspect that var. *laxa* was based on the *lyallii* × *odontoloma* hybrid, which does occur there (Figs. 2, 3). The description of the leaf petiole and lamina suggests this hybrid, but, unless authentic material should prove to have survived, var. *laxa* is best regarded as a *nomen dubium*.

The population of *Saxifraga lyallii* from Alaska to southern British Columbia (var. *hultenii*) is extremely uniform. It now seems probable that the occurrence of two geographically separate rusts on this population is due to one rust having spread southward more slowly than the plants travelled and the other having spread northward, from *S. odontoloma* or *S. lyallii* var. *lyallii* in the Cascades, after one of these plants met *hultenii*.

FIG. 3. Distribution of *Saxifraga lyallii* var. *lyallii* and var. *hultenii*.

S. LYALLII var. LYALLII

ALBERTA: Beehive Mtn., 30 miles north of Crowsnest Pass, *Mountjoy 38* (DAO); Crowsnest Pass, Aug. 2, 1897, *John Macoun* (CAN). Waterton Lakes National Park: near Crypt Lake, *Moss 10512* (CAN), ditto, *Senn 2652* (DAO); Cameron Lake, *Taylor 9685* (UBC); Lake Alderson, *Carmichael 71* (DAO, in part). Banff National Park: Mt. Norquay, *Calder & Savile 12221* (DAO), July 29, 1915, *Pelluet* (CAN), Aug., 1916, *Lewis* (CAN), *Jenkins 1587* (DAO); Mt. Louis, Aug. 9, 1916, *Lewis* (CAN); Mt. Aylmer, Aug. 6, 1891,

*John Macoun* (CAN); Saddle Mtn., Aug. 4, 1891, *John Macoun* (CAN); Lake Agnes, Aug. 19, 1891, *John Macoun* (CAN); Helen Lake, *Mair 245* (UBC). Opal Hills near Maligne Lake, Jasper National Park, *Jenkins 7925* (DAO). BRITISH COLUMBIA: Berg Lake, Mt. Robson Provincial Park, *Jenkins 7166* (DAO); Paradise Mine near Windermere, *Calder & Savile 11236* (DAO); Twin Falls, Yoho National Park, July 26, 1927, *Bostock* (DAO, in part); Little Yoho River, Yoho National Park, *Bostock* (DAO); Maus Creek, Fort Steele, Aug. 5, 1942, *Johnstone* (V); Grizzly Gulch near Starvation Peak, 49° 02′ N. 114° 11′ W., *Taylor, Calder, & Ferguson 3436* (DAO); Wall Lake, B.C.–Alberta border, *Taylor 8965* (UBC), *Taylor, Calder, & Ferguson 3487* (DAO); Bald Mtn., Glacier National Park, *Fogg 1238* (UBC); Cheam Range, Aug. 10, 1901, *J. M. Macoun* (CAN); Tamihi (Tami Hy) Mtn., Chilliwack Valley, Aug. 28, 1901, *J. M. Macoun* (CAN, in part); second summit west of Skagit River, July 28, 1905, *J. M. Macoun* (CAN); Lake O'Hara, Aug. 10, 1904, *John Macoun* (CAN); Kinbasket Mtn., 52° 00′ N. 118° 01′ W., *Taylor & Ferguson 3683* (DAO); Nub Mtn., Mt. Assiniboine Park, *Taylor & Ferguson 3795* (DAO); Tornado Mtn., 49° 58′ N. 114° 38′ W., *Taylor & Ferguson 3167* (DAO). MONTANA: Logan Pass, *Porsild & Johannes 19729* (CAN); Sperry Glacier, *Vreeland 1091* (CAN); Grinnell Glacier trail, *Jones 5461* (WTU); Iceberg Lake, Mt. Wilbur, *Brant 141* (WTU); Mission Range SE. of MacDonald Lake, Lake Co., *Hitchcock 18298* (WTU); Pintlar Peak, Anaconda Range, Beaverhead Co., *Hitchcock & Muhlick 12865* (WTU). WASHINGTON: below Slate Peak, Okanogan Co., *Ownbey & Meyer 2301* (WTU); Mt. Herman, Whatcom Co., *Thompson 5713, 8045* (WTU); Mt. Baker, Whatcom Co., *Thompson 11242* (WTU); Church Mtn., Whatcom Co., *Thompson 11282* (WTU, in part).

2*b*. **Saxifraga lyallii** Engler var. **hultenii** Calder & Savile var. nov.

Folia flabellata, glabra vel paullulum pilosa, majores ad 2.5–8.5(–11.5) cm long. petiolis inclusis, 1.5–4.5(–6.5) cm lat., plerumque abrupte contracta, dentibus 7–11(–14) in arcu apicali positis. Scapi 9.0–30.0 cm alt., ad basas glabri vel rariter paullum pilosi, ad summas glandibus paucis vel numerosis atropurpureis capitatis instructi; inflorescentia 3.0–15.0 cm long., 1.0–5.0(–8.0) cm lat. Sepala ca. 1.8–3.8(–4.4) mm long.; petala 2.4–4.8 mm long., 1.4–3.0 mm lat., plerumque conspicue ungulata, verisimiliter semper luteo–vel viridimaculata; discus saepe apparet in basa capsulae ut cingulum pallidum albomaculatum; capsulae 6.5–11.0 mm long., (2.0–)3.2–5.0(–7.5) mm lat.

*Type*.—Twenty miles south of Moresby Camp, Moresby I., Queen Charlotte Is., B.C., *Calder, Savile, & Taylor 23106* (DAO).

*Range*.—Alaska Peninsula and central Alaska, eastward across Yukon to Mackenzie border, southward to Whatcom Co., Wash., and nearly to Crowsnest Pass, Alta. (Fig. 3).

We are pleased to dedicate this variety to Prof. Eric Hultén, whose studies of the Flora of Alaska provided much of the basis for the work reported in this paper.

This widespread and relatively common variety is substantially taller than var. *lyallii* and has larger and generally distinctively fan-shaped leaves.

It may be noted that the plant recorded and illustrated from southwestern Yukon by Löve and Freedman (5) as *Saxifraga lyallii* is actually *S. davurica* Willd. ssp. *grandipetala* (Engl. & Irmsch.) Hult., which is already recorded from Yukon. It is distinguishable from *S. lyallii* by its elliptical and unspotted petals, short capsules, lack of capitate glands on the panicle branches, and, from var. *hultenii*, by the generally slender and spatulate leaves. The long leaves and rather short scapes give the plant a distinctive appearance. It may be noted that the key characters used by Engler and Irmscher to distinguish *S. lyallii* and *S. davurica* are valueless at least for *S. davurica* ssp. *grandipetala*. In this taxon the sepals seem to reflex just as promptly as in *S. lyallii* and the filaments may vary from subulate to clavate in a single plant.

YUKON: Cassiar Mts., *Poole 61* (DAO), 1952, *Poole* (DAO); Mile 84, Haines Road, *Clarke 505* (CAN); Mile 95, 118, 240–250, and 268, Canol Road, *Porsild & Breitung 11935, 10133, 11433, 11356* (CAN); MacMillan Pass, Canol Road, *Porsild & Breitung 11240* (CAN); Mt. Sheldon, Canol Road, *Porsild & Breitung 10133* (CAN). ALASKA: Chugach Mts., Anchorage, *Dutilly et al. 21139* (DAO, CAN), Aug. 21, 1936, *E. & J. Lohbrunner* (V); Juneau, *Anderson 6269* (DAO, CAN); Ketchikan, *Walkers 979A* (DAO, in part); near Divide, Rocky Creek (Kenai Lake), Moose Pass Valley, Mt. Marathon (Seward), near Lawing, near Hope, Stetson Creek Valley (all Kenai Peninsula), *Calder 5481, 5601, 5753, 5877, 6161, 6327, 12268* (DAO); Willow Creek Road, Talkeetna Mts., *Anderson 1025* (CAN); White Pass, *Malte 308* (CAN); Tangle Lake, west of Paxson, *Gjaerevoll 1231* (CAN). ALBERTA: Agnes Lake near Lake Louise, Banff National Park, *Beattie 5274* (DAO); Laggan, July 11, 1913, *Malte* (CAN); near Highwood Pass south of Kananaskis, *Moss 10738* (CAN); Pabocton Creek, Jasper National Park, *Brown 1118* (CAN); mountains north of Banff, July 5, 1900, *Sanson* (CAN); Blair Mtn., July 23, 1923, *Lambart* (CAN); Lake Louise, Aug. 8, 1898, *Anderson & Fletcher* (V), July 30, 1904, *John Macoun* (CAN); Sulphur Mtn., Banff, July 18, 1891, *John Macoun* (CAN); Moose Mtn., Elbow River, July 1, 1897, *John Macoun* (CAN). BRITISH COLUMBIA: Bluff Lake area 16 miles SW. of Tatla Lake P.O., *Calder, Parmelee, & Taylor 19319, 19335* (DAO); Mt. Arrowsmith, *Calder, Parmelee, & Taylor 19464* (DAO), Sept. 21, 1938, *Cowan* (V), Aug. 16, 1929, *Pool* (V); between Jesmond and Kelly Lake, *Calder, Parmelee, & Taylor 18723* (DAO); Foghorn Mtn. near Chua Chua, Aug. 8, 1936, *Tisdale* (DAO); near Griffin Lake, Aug. 7, 1889, *John Macoun* (DAO, CAN); Mile 46 and 68 Haines Road, *Taylor et al. 1564, 903* (DAO); Cassiar, *Taylor et al. 595* (DAO); 20 miles south of Moresby Camp, Moresby I., Queen Charlotte Is., *Calder, Savile, & Taylor 23106* (DAO, type); mountain west of Mosquito L., Moresby I., Queen Charlotte Is., *Calder & Taylor 23696* (DAO); Mt. Thornhill near Terrace, *Calder, Savile, & Ferguson 13323, 14857* (DAO); Red Rose Tungsten Mine near Skeena Crossing, *Calder, Savile, & Ferguson 15141, 15164* (DAO); Sylvan Mine near Smithers, *Calder, Savile, & Ferguson 12953, 14604* (DAO); Murray Range at Pine Pass along Hart Highway, *Calder, Savile, & Ferguson*

*14032* (DAO); Glacier, Aug. 7, 1899, *Fletcher* (DAO); Mt. Revelstoke National Park, *Calder, & Savile 11865* (DAO); Cariboo Hudson Mine SE. of Barkerville, *Calder, Savile, & Ferguson 14228* (DAO); Wolverine Range near Manson Creek, *Calder, Savile, & Ferguson 13695* (DAO); Atlin Lake area, *Aitken 22* (DAO); Mt. Stephen, Field, *Calder & Savile 12082* (DAO); Bennett, *Mitchell 142* (DAO, UBC); Omineca Mts., July 26, 1941, *Fletcher* (V); three miles WSW. of Donald, *Taylor & Ferguson 1855* (DAO); Lytton Mts., July 14, 1924, *Anderson* (V); Alice Arm, *Lohbrunner & Nichols* (V); Goat Mtn., 49° 58′ N. 114° 42′ W., *Taylor & Ferguson 3063* (DAO); Mt. McLean, Lillooet, *Calder, Savile, & Ferguson 15509* (DAO); trail to Floe Lake, Kootenay National Park, July 16, 1932, *Hume* (UBC); Mt. Waddington, July, 1937, *Taylor* (UBC); Little Yoho Valley, *Brown 452* (CAN); Mt. Selwyn, *Raup & Abbe 3786, 4152* (CAN); Mt. Brilliant, Rainbow Mts., *Laing 541* (CAN); near Yukon border at 136° 58′ W., *M.P. & R.T. Porsild 250* (CAN); Sheep Creek and Jack Pine Pass, continental divide, *Lambart 68, 69* (CAN); Chipuin Mtn., Marble Mts., *J. W. & E. M. Thompson 628* (CAN); Rogers Pass, Selkirk Mts., Aug. 2, 1890, *John Macoun* (CAN); Kicking Horse Pass, Sept. 13, 1884, *John Macoun* (CAN). WASHINGTON: Church Mtn., Whatcom Co., *Thompson 11282* (WTU, in part).

Atypical S. LYALLII var. HULTENII

BRITISH COLUMBIA: Garibaldi Provincial Park: Aug. 2, 1914, *Davidson* (DAO); July 28, July 29, Aug. 6, 1926, *Hardy* (V); *Cottle 370* (UBC); July, 1913, *Perry* (UBC); Aug. 8, 1938, *Eastham* (DAO); Aug. 12, 1938, *Eastham* (UBC). Mt. Arrowsmith, Aug. 12, 1917, *Kermode* (V).

Intermediate between S. LYALLII var. LYALLII and var. HULTENII

ALBERTA: Waterton Lakes National Park: Cameron Lake, *Taylor 9683* (UBC); Crypt Lake, *Moss 10512* (DAO). BRITISH COLUMBIA: Paradise Valley, July 15, 1923, *Anderson* (V); Green Mtn., near Haylmore, *J. W. & E. M. Thompson 646* (CAN, DAO); seven miles NE. of Natal near Alexander Creek, *Taylor & Ferguson 2289* (DAO); 16 miles east of Waldo, *Taylor & Ferguson 3196* (DAO).

*3a.* SAXIFRAGA PUNCTATA L., Sp. Pl., ed. 1, 401. 1753, ssp. PUNCTATA

Leaves reniform to orbicular, the largest 4.5–6.5 cm wide with ca. 20–24 teeth usually deeply cordate, sinus occasionally closed, teeth up to 1.0 cm long and wide, mammiform or rarely triangular, overlapping or open, (glabrous) glabrate to sparsely pubescent with white hairs; petioles up to 9.5 cm long, usually glabrous below, often sparsely to moderately pubescent toward blade. Scapes 19.0–42.0 cm high, glabrate to sparingly pubescent below, becoming moderately short-glandular pubescent above, the hairs nearly all tipped with yellowish or reddish glands; panicles open, 3.0–12.0(–17.0) cm long, 2.0–6.5 cm wide, branches ascending or spreading, slender, sometimes capillary, with pubescence as in upper part of scape; pedicels 0.15–0.25 mm diameter. Sepals (0.7–)1.0–1.5 mm long, 0.5–1.0 mm wide, usually green,

occasionally reddish or reddish-margined, glabrous or occasionally with a few glands and minutely ciliolate; petals 2.0–3.5(–4.0) mm long, 1.1–1.8 mm wide, elliptic to ovate, tapering to a narrow base or slightly clawed, white, midvein generally obscure; filaments (1.5–)2.0–2.8 mm long, 0.25–0.45 mm wide, strongly clavate or occasionally elliptic with a slender base, white; disk seldom conspicuous even in flower; stigma 0.15–0.3 mm diameter; capsule 4.0–4.8 mm long, 1.7–3.0 mm wide, cleft 30%–75% of its length, with beaks slender and strongly divergent, green to purplish red.

*Type* (*not seen*).—Siberia (LINN).

*Range.*—Ural Mts. to eastern Siberia.

*Saxifraga punctata* ssp. *punctata* does not reach our area, but a full description has been included here as a basis of comparison with the North American populations of the species. It intergrades with ssp. *nelsoniana* in extreme eastern Siberia.

The synonymy of some of the subspecies of *S. punctata* is involved. In the following treatment no attempt is made to record all the epithets that have been erroneously applied to these plants.

Hultén (4) was the first to treat the New World subspecies of *S. punctata* on a realistic basis, but the material available to him from beyond the limits of Alaska was inadequate; and, in consequence, he overestimated the southward extent of ssp. *pacifica* and the eastward extent of ssp. *nelsoniana*. We have found it necessary to recognize three additional subspecies; one of these, ssp. *cascadensis*, was clearly suspected by Prof. Hultén, to judge by his annotation of a sheet seen by us, and his published statement.

*3b.* SAXIFRAGA PUNCTATA L. ssp. NELSONIANA (D. Don) Hult., Flora of Alaska and Yukon, 5: 929, 1945.

*Saxifraga nelsoniana* D. Don, Trans. Linn. Soc. 13: 355. 1822.

*Saxifraga punctata* L. var. *nelsoniana* (D. Don) Macoun, Cat. Can. Pl. 1: 153. 1883.

*Micranthes nelsoniana* (D. Don) Small, N. Am. Fl. 22, 2: 147. 1905.

Largest leaves 1.8–5.0(–6.0) cm wide with (13–)17–21(–25) teeth, shallowly to deeply cordate, sinus rarely closed; teeth up to 0.7(–1.2) cm long, 1.0 cm wide, nearly always mammiform; lightly to densely short pubescent; petioles up to 9.5 cm long, lightly pubescent below, densely so near blade. Scapes 7.0–36.0 cm high, slightly to moderately long pubescent below, becoming densely short or glandular-pubescent above, the hairs obscurely septate; panicles usually congested, occasionally with one distant basal branch, 0.8–3.5(–10.0) cm long, 1.2–4.0 cm wide, branches stout; pedicels 0.2–0.3 mm diameter. Sepals 1.1–2.1 mm long, 0.6–1.2 mm wide, green to purplish red, glandular and often ciliolate; petals 3.0–4.5 mm long, 1.4–2.5 mm wide, elliptic to broadly ovate, short clawed, white; filaments 1.9–3.7 mm long, 0.2–0.4 mm wide, usually strongly clavate, base generally filiform, nearly always white; disk conspicuous in flower, often visible in fruit; stigma 0.25–0.5 mm diameter; capsule 3.0–6.5 mm long, 2.0–4.0 mm wide, cleft 20%–70% of its length; beaks moderately slender and moderately divergent.

FIG. 4. Distribution of the North American subspecies of *Saxifraga punctata*.

*Type* (*not seen*).—Cape Newenham, Alaska (K); photograph seen.

*Range.*—Extreme eastern Siberia, eastward to Mackenzie R. delta, southward in interior Alaska and extreme western Yukon north of the coast ranges (Fig. 4).

Its strongly pubescent leaves and densely glandular inflorescence make ssp. *nelsoniana* a distinctive plant, but it intergrades freely with sspp. *punctata*, *insularis*, *pacifica*, and *porsildiana*. The plants from the type region are not so densely pubescent as those from further north and northeast, and are suspected of tending slightly to ssp. *insularis* or ssp. *pacifica*.

MACKENZIE DISTRICT: Reindeer Station, Mackenzie Delta, *Porsild 16756* (CAN); east slope of Mackenzie Mts., west of Mackenzie Delta, *Porsild 6686, 6824* (CAN). YUKON: King Point, June 20 – July 4, 1906, *Lindstrom* (NY), June–July, 1906, *Hansen* (CAN); between King and Kay Point, *Porsild 7146* (CAN); Shingle Point, *Cox & O'Neill 174, 174a* (CAN), *Porsild 6729, 7107* (CAN); Herschel Island, *Johansen 217* (CAN, NY), 1893, *Stringer* (CAN); 65° 58′ N. 141° 00′ W., June 29, 1912, *Cairnes* (CAN); Yukon–Alaska border west of Dawson, *Calder & Billard 3621* (DAO); 60 Mile Road between Dawson and the Alaska border, *Calder & Billard 3353, 3747, 3748, 4474* (DAO); Miller Creek, June 28, 1917, *Cairnes* (CAN); 24 Mile House, Dawson Area, *Eastwood 381* (CAN). ALASKA: Hall Island, *Trelease 4053, 4054* (DAO); St. Matthew Island, *Trelease 4058* (DAO); St. Lawrence Island, July 22, 1952, *Fay* (UBC), July 10, 1953, *Fay* (CAN), Aug. 13, 1891, *J. M. Macoun* (CAN); King Island, *McIlhenny 68* (NY); Little Diomede Island, *A. E. & R. T. Porsild 1704* (CAN); Camden Bay, *Johnsen 70, 99* (CAN); Pt. Hope, *Rasmussen 1339* (CAN), July, 1884, *Yemans* (NY); Point Barrow, *McIlhenny 70* (CAN, NY), *Dutilly et al. 21703* (CAN), *Spetzman 2403* (CAN); Cape Nome, 1900, *Blaisdell* (NY); Nome, June 9, 1951, *Whillans* (DAO), *Dutilly et al. 20764* (DAO); 35 miles south of Point Barrow, *McIlhenny 71* (NY); Port Clarence, Seward Peninsula, *Trelease 4055, 4056* (DAO); Umiat, *Lepage 23611* (CAN), *Dutilly et al. 21778* (CAN), *Spetzman 1359, 2332* (CAN); Alaktak, *Scholander 342* (DAO); *Spetzman 2485* (CAN); Lake Schrader, *Spetzman 795* (CAN); Barter Island, *Spetzman 1206* (CAN); Anaktuvuk Pass, *Spetzman 1698, 1845* (CAN); Sadlerochit River, *Spetzman 853, 1130* (CAN); Mile 112 Steese Highway, *Cody & Webster 5386* (DAO); Eagle Summit, Aug. 4–10, 1936, *E. & J. Lohbrunner* (CAN, V); head of Chatanika River, *Lepage 23300* (DAO); Upper Kurupa River Valley, *Hodgdon 8223* (DAO); Wonder Lake, Mt. McKinley Nat. Pk., *Dutilly et al. 20349* (DAO); Ogilvie Mts., July 16–27, 1936, *E. & J. Lohbrunner* (V); White Mts., *Gjaerevoll 283B* (CAN); Wiseman, *Jordal 2129* (CAN); Healy, *A. E. & R. T. Porsild 356* (CAN); between Summit and McCarty, *A. E. & R. T. Porsild 428* (CAN); Cantwell, *A. E. & R. T. Porsild 55* (CAN); Teller, *A. E. & R. T. Porsild 1438* (CAN).

3*c*. **Saxifraga punctata** L. ssp. **carlottae** Calder & Savile ssp. nov.

Laminae foliorum orbiculares vel reniformes, truncatae vel subcordatae, majores ad 3.5–5.2(–5.7) cm lat., dentibus mammiformibus 9–12 usque ad 1.2 cm alt. et lat., modice pubescentibus pilis brevibus alboluteis sed in

margine et versus petiolum pilis longioribus; petioli ad 6.5–7.8 cm long., ad apicem flabbellati, modice lanato-pubescentes. Scapi 11.0–28.0(–33.0) cm alt., ad basas modice pubescentes, ad summas dense lanato-pubescentes pilis inconspicue glandulosis glandibus subrufis; paniculae florentes ramibus laxis (1.2–8.0 cm alt., 1.2–4.8 cm lat.), maturescentes plerumque strictis (1.6–10.5 (–12.0) cm alt., 0.8–3.0(–5.0) cm lat.), robustis; pedicelli 0.25–0.5 mm diam. Sepala 1.4–2.4 mm long., in fructu 2.0–3.5 mm long., 0.4–1.2 mm lat., margine subrufa, saepe glanduliciliolata; petala 3.0–5.5(–6.0) mm long., 1.0–2.4(–2.6) mm lat., ab ellipticis ovata, ad basas cuneata vel subunguiculata, ab albis roseotincta; filamentia 2.5–4.2 mm long., 0.15–0.45(–0.6) mm lat., a filiformibus clavata, ad basas graciles, alba vel rubescentia; discus inconspicuus; stigmata 0.25–0.5 mm diam.; capsulae 5.5–12.0 mm long., 2.0–4.5 mm lat. (usque ad 6.0 mm lat. fructibus 3–4-carpellatis), bifidae ad 70–90% sed sinu clauso et rostris tantum paullum divaricatis, purpureo-rubra vel interdum virides.

*Type.*—Twenty miles south of Moresby Camp, Moresby I., Queen Charlotte Is., B.C., *Calder, Savile, and Taylor 23093* (DAO).

*Range.*—Queen Charlotte Is., B.C., and adjacent mainland (Fig. 4).

This subspecies is chiefly distinguished by the scarcely cordate leaves with usually less than 12 teeth, and the long, narrow, deeply cleft capsules. The capsule valves are often so closely appressed that the degree of cleavage may not be noticeable to the unaided eye. Subspecies *carlottae* is abundant on moist alpine or subalpine slopes or clefts in the Queen Charlotte Islands; but it has also spread to the mainland where it intergrades freely with ssp. *pacifica* and ssp. *porsildiana*. Its relationship with ssp. *cascadensis* is discussed under that taxon.

Attention must be drawn to the plants in the Prince William Sound region of southern Alaska, cited as intermediate between ssp. *carlottae* and ssp. *pacifica*. These plants are actually closer in morphology to *carlottae* than to *pacifica*, despite the occurrence of pure *pacifica* further eastward. This population will be discussed under Phytogeography.

ALASKA: Deer Mtn., Ketchikan, *Walkers 979B* (DAO). BRITISH COLUMBIA: QUEEN CHARLOTTE ISLANDS: head of McClinton Bay, Masset Inlet, Graham Island, *Calder, Savile, & Taylor 21617* (DAO); 20 miles south of Moresby Camp, Moresby Island, *Calder, Savile, & Taylor 23093* (DAO, type); Shields Bay, Rennell Sound, Graham Island, *Calder & Taylor 23340* (DAO); below Mt. de la Touche, Fairfax Inlet, Tasu Sound, Moresby Island, *Calder & Taylor 23513* (DAO); west end of Mosquito L., Moresby Island, *Calder & Taylor 23702* (DAO). Stuie, Caribou Mts., *Laing 543* (CAN); Prudhomme L. near Prince Rupert, *Taylor & Lewis 638* (UBC).

S. PUNCTATA ssp. CARLOTTAE – ssp. PORSILDIANA

BRITISH COLUMBIA: Sylvan Mine, 9 miles west of Smithers, *Calder, Savile, & Ferguson 14605* (DAO); Red Rose Tungsten Mine, east of Skeena Crossing, *Calder, Savile, & Ferguson 13363, 15164A, 15165* (DAO); Glacier Gulch, Hudson Bay Mtn., Smithers, *Calder, Savile, & Ferguson 12895* (DAO).

3*d*. **Saxifraga punctata** L. ssp. **cascadensis** Calder & Savile nom. nov.

*Saxifraga paddoensis* Suksdorf, W. Am. Sci. 15: 59. 1906.

*Saxifraga aestivalis* auct. p.p., non Fisch. & Mey., Ind. Sem. Hort. Petrop. 1:37. 1835.

*Micranthes aestivalis* sensu Small, N. Am. Fl. 22, 2: 147. 1905, p.p.

Laminae foliorum orbiculares, leviter vel valide cordatae, raro sinu clauso, majores ad 2.0–5.7 cm lat., dentibus (14–)16–19(–22) usque ad 1.0(–1.3) cm long, et 1.0(–1.2) cm lat., glabrae vel glabratae, interdum praecipue dentes ciliolatae; petioli usque ad 14.0 cm long., a glabratis leniter pubescentes. Scapi 5.0–33.0 cm alt., ad basas glabri vel leniter pubescentes, ad summas pubescentes pilis appressis eglandulosis vel tantum inconspicue glandulosis; paniculae 1.2–11.5 cm long., 1.2–4.5(–8.0) cm lat., ramis plerumque adscendentibus subcapillaribusque; pedicelli 0.1–0.3 mm diam. Flores delicatuli; sepala 0.8–1.6 mm long., in fructu usque ad 2.2 mm long., 0.5–0.8 mm lat., margine plerumque subrufa, saepe glanduliciliolata; petala 2.1–3.2 mm long., 0.9–1.8 mm lat., alba vel raro primo roseotincta; filamenta 1.6–3.1(–3.7) mm long., 0.1–0.2(–0.3) mm lat., a filiformibus anguste clavata vel interdum elliptica, ad basas elliptica, alba vel raro subrufa; discus in statu florendi conspicuus, in fructus tantum ut cingulum albomaculatum in basa capsulae apparet; capsulae 3.3–6.4 mm long., 1.5–3.3 mm lat., bifidae ad 30–50%, rostris leviter vel modice divaricatis.

*Type*.—Mt. Paddo (Mt. Adams), Yakima Co., Wash., *Suksdorf 2504* (NY).

*Range*.—Southwestern B.C., western Wash., and northwestern Ore. (Fig. 4).

Hultén (p. 932) has already pointed out that Fischer and Meyer, under the impression that *Saxifraga punctata* was a plant resembling *S. davurica*, applied the name *S. aestivalis* to plants of *S. punctata* from Altai, Yenisei, Kamtchatka, and Sitka. *S. aestivalis* is thus a synonym of *S. punctata* s. lat.; and the name cannot be applied to any subspecies of it, least of all the one under discussion.

One name has been validly applied to this subspecies, *S. paddoensis* Suksdorf. However, that name has subsequently been submerged in the confusion attending the plants of this complex. Because this taxon is definitely not a full species, we have availed ourselves of the privilege of selecting for it a new epithet, which, incidentally, denotes its main range.

*Saxifraga punctata* ssp. *cascadensis* somewhat resembles ssp. *punctata* in its small flowers and slender panicle branches and pedicels; but it differs in the pubescence of the leaves and particularly scapes, and in the slenderness of its filaments. It is more sharply distinct from the other North American subspecies. It has invaded the Queen Charlotte Islands where it intergrades with ssp. *carlottae* (see under Phytogeography).

BRITISH COLUMBIA: Hope-Princeton Highway, Manning Park, *Calder & Savile 10607A* (DAO); Blackwall Peak, Manning Park, *Calder & Savile 11594* (DAO); Mt. McLean near Lillooet, *Calder, Savile, & Ferguson 15507* (DAO), July 19, 1916, *J. M. Macoun* (NY, CAN); Mt. Cheam, July 30, 1898, *Fletcher* (DAO), Sept. 5, 1954, *Brayshaw & Szczawinski* (UBC, DAO), Sept. 13, 1916, *Perry* (UBC), *Fletcher & Anderson 871* (V); Garibaldi Park, Aug.

1914, *Davidson* (DAO), Aug. 10, 1912, *Davidson* (UBC), Aug. 8, 1938, *Eastham* (DAO), Aug. 9 & 10, 1938, *Eastham* (UBC); Alta Lake, Aug. 1916, *Perry* (UBC); Dam Mtn., 1933, *Taylor* (UBC); Sproat Lake, Vancouver Island, *Taylor* 1231 (UBC); Forbidden Plateau, Vancouver Island, Aug. 17, 1950, *Carter* (UBC); Strathcona Park, Vancouver Island, Aug. 30, 1938, *Slocomb* (V); Tsable Mtn., Vancouver Island, Aug. 10, 1943, *Wyatt* (V); Canim Mine, Hope-Princeton Highway, July, 1949, *Hardy* (V); Timberline Valley, Manning Park, Aug. 2 & 5, 1947, *Hardy* (V); McGillivray Creek, Cascade Range, Aug. 10, 1916, *J. M. Macoun* (CAN, NY), Aug. 12, 1916, *J. M. Macoun* (NY, CAN, V); Lake House, Skagit River, July 1, 1905, *J. M. Macoun* (CAN, NY); second summit west of Skagit River, July 21, 1905, *J. M. Macoun* (NY, CAN); Tulameen River, 1900, *Kemp* (NY); Chilliwack Valley, July 8, 1901, *J. M. Macoun* (NY, in part), July 20, 1901, *J. M. Macoun* (NY), July 22, 1901, *J. M. Macoun* (NY); Chilliwack Lake, July 8, 1901, *J. M. Macoun* (CAN); 20 miles south of Moresby Camp, Moresby Island, Queen Charlotte Islands, *Calder, Savile, & Taylor 23113* (DAO); west end of Mosquito L., Moresby Island, Queen Charlotte Islands, *Calder & Taylor 23763* (DAO). WASHINGTON: Mt. Stuart, Chelan Co., *Sandberg & Leiberg 570* (NY); Cascade Tunnel, Chelan Co., July 14, 1911, *Jones* (NY); Olympic Mts., Clallam Co., *Elmer* 2640 (NY); Olympic Mts., *Piper 2214* (NY); Baldy Peak, Grays Harbour Co., *Lamb 1342* (NY); Marmot Pass, Jefferson Co., *Thompson 9884* (NY); Sawtooth Ridge, Mason Co., *Meyer 1705* (NY); Bridge Creek, Okanogan Co., *Elmer 716* (NY); Van Trump Park, Mt. Rainier, Pierce Co., *Thompson 12552* (NY); Paradise Valley, Mt. Rainier, Pierce Co., *Jones 8109, 8166, 8180* (NY); Mt. Rainier, Pierce Co., *Piper 2040* (NY); Mt. Pugh, Snohomish Co., *Thompson 14312* (NY); Mt. Paddo (Mt. Adams), Yakima Co., *Suksdorf 2504* (NY, type); Chinook Pass, Yakima Co., *Thompson 15097* (NY); Mt. Baker, Whatcom Co., *Thompson 11013, 11257* (NY); Wellington, *Umbach 551* (NY); Goat Mts., *Allen 198* (NY, CAN). OREGON: Mt. Jefferson, Linn Co., *Peck 9107* (NY).

*3e.* SAXIFRAGA PUNCTATA L. ssp. PACIFICA Hult., Fl. Alaska and Yukon, 5: 928. 1945.

? *Saxifraga arguta* D. Don, Trans. Linn. Soc. 13: 356. 1822.

? *Saxifraga aestivalis* Fisch. & Mey., Ind. Sem. Hort. Petrop. 1: 37. 1835.

? *Micranthes aestivalis* (Fisch. & Mey.) Small, N. Am. Fl., 22, 2: 147. 1905.

Largest leaves 2.3–5.7(–7.7) cm wide with usually 14–18 teeth, shallowly to deeply cordate, slightly ciliate, otherwise glabrous or a few hairs at junction of petiole and blade; petioles up to 12.5(–17.5) cm long, glabrous or with a few hairs near blade. Scapes 11.0–33.0(–38.0) cm high, lightly to moderately pubescent below, becoming more or less densely pubescent and glandular above, the hairs usually obscurely septate; panicles open to moderately congested, 1.2–15.0(–19.0) cm long, 1.5–4.0(–6.5) cm wide, branches stout; pedicels 0.2–0.4 mm diameter. Sepals 1.7–2.5 mm long, 0.7–1.0 mm wide, green to purplish red, usually glandular and ciliolate; petals 2.0–3.5 mm

long, 1.0–1.5(–2.3) mm wide, elliptic to broadly ovate, usually short-clawed, white, rarely red-tinged; filaments (1.5–)2.3–2.8 mm long, 0.1–0.4 mm wide, filiform to strap-shaped, elliptic or clavate, rarely triangular, generally white; disk visible in flower, obscure in fruit; stigma 0.2–0.4 mm diameter; capsule 4.0–8.0 mm long, (2.2–)3.0–4.5 mm wide, cleft 25%–60% of its length, beaks moderately slender and slightly to moderately divergent.

*Lectotype.*—Juneau, *Anderson 6270* (S). Isotypes CAN, DAO.

*Range.*—Southern Alaska from Kodiak I. intermittently nearly to the southeast tip of the Alaska panhandle (Fig. 4).

*Saxifraga punctata* ssp. *pacifica* intergrades freely with sspp. *insularis*, *nelsoniana*, and *carlottae* wherever it meets them. Its range is much more restricted than Hultén supposed, for it is displaced in the southern part of the Alaska panhandle by ssp. *carlottae* and ssp. *porsildiana*. It has not spread eastward across the coast mountains in British Columbia, although it will be seen, from Fig. 4, to have spread sparingly northward into the interior of Alaska. Despite its narrow and somewhat intermittent range it must be regarded as a valid subspecies, for it is not intermediate in morphology between any two neighboring populations. The plants in the Alaska panhandle that Hultén believed, on the basis of leaf pubescence, to be grading toward *nelsoniana* are actually intermediate between *pacifica* and *carlottae*, as is clear from both leaf and capsule shape.

For the identity of *Saxifraga arguta* see discussion under *S. odontoloma*.

ALASKA: Juneau, Aug. 6, 1891, *Cooley* (NY), *Anderson 487* (NY), *Trelease 4061* (DAO), *Anderson 6270* (S, type; CAN, DAO); vicinity of Yakutat Bay, *Funston 40* (CAN, NY); Yes Bay, *Gorman 66* (NY); Skagway, *Anderson 798* (NY); Windham Bay, *Culbertson 4888* (NY, in part); Karluk, Kodiak Island, *Rutter 108* (NY); Three Saints Bay, Kodiak Islands, *Eyerdam 338*, *388* (S); Port Vita, Raspberry Island, Kodiak Islands, *Eyerdam 3092* (DAO), *3760* (DAO, S), *3937* (S); Kukak Bay, *Saunders 4065* (DAO); Port Hobron, Silkalidak I., Kodiak Islands, *Eyerdam 46*, *538* (S); Earl River near Mt. McKinley, July 27, 1903, *Shainwald* (NY); Port San Juan, Evans Island, *Eyerdam 7076* (DAO, CAN), *Eyerdam 5864* (CAN, S); Thum Bay, Knight Island, Prince William Sound, *Eyerdam 3362* (DAO); Seward, 1908, *Diehl* (NY), *Calder 5287* (DAO); Stetson Creek Valley, Kenai Peninsula, *Calder 6496*, *6500* (DAO); Rocky Creek, Kenai Lake, Kenai Peninsula, *Calder 5605* (DAO); Orca, *Trelease 4050* (DAO); Disenchantment Bay, *Coville & Kearney 1081* (CAN); Talkeetna Mts., Aug. 9, 1931, *Anderson* (CAN), *Anderson 1001* (S); White Pass, *Malte 306* (CAN); between Chickaloon and Alfred Creek, *Pope 69* (S); Orca, Prince William Sound, June 14, 1937, *Norberg*, (S). BRITISH COLUMBIA: Mile 45 and 46 Haines Road, *Taylor et al. 1511*, *1520* (DAO).

S. PUNCTATA ssp. PACIFICA – ssp. CARLOTTAE

ALASKA: Orca, Prince William Sound, Aug. 6, 1938, Norberg (S, in part); Hinchinbrook I., *Steenis 4808* (S); Thum Bay, Knight Island, Prince William Sound, *Eyerdam 3367* (DAO); Port San Juan, Evans I., *Eyerdam 7128* (CAN, S); Granite Creek, *Cowles 1365* (DAO); Shepard Point Cannery, near Cordova,

July 30, 1936, *Baxter* (DAOM); Aqua Dulce River, Yakutat Bay, *Trelease 4048* (DAO); Hyder, *Anderson 5519* (CAN); Sitka, *Hultén 8390* (S); Juneau, *Hultén 8269, 8274* (S); Yes Bay, *Howell 1622* (NY); Windham Bay, *Culbertson 4888* (NY, in part). BRITISH COLUMBIA: Alice Arm, July, 1934, *Lohbrunner & Nichols* (V, DAO); Mt. Thornhill, Terrace, *Calder, Savile, & Ferguson 14841* (DAO); near Tyee on Prince Rupert – Terrace road, *Calder, Savile, & Ferguson 15046* (DAO); near junction of Legate Creek and Skeena River, *Calder, Savile, & Ferguson 14814* (DAO).

S. PUNCTATA ssp. PACIFICA – ssp. CARLOTTAE – ssp. PORSILDIANA

BRITISH COLUMBIA: Mt. Thornhill, Terrace, *Calder, Savile, & Ferguson 13307* (DAO).

S. PUNCTATA ssp. PACIFICA – ssp. NELSONIANA

ALASKA: Curry, *Schofield 1846* (DAO); Hope, 3 miles north of Seward, Divide, Sixmile Creek, Falls Creek near Lawing, and Palmer Creek, all Kenai Peninsula, *Calder 5234, 5251, 5318, 5956, 6160, 6328* (DAO); Tetlanika River, Mt. McKinley Nat. Pk., *Mexia 2041* (NY); Mile 189 Richardson Highway, *Webster 118* (DAO); Mile 213 & 301 Richardson Highway, *Cody & Webster 5206, 5521* (DAO); Mile 118 Paxton–Cantwell Highway, *Webster 235* (DAO); Chugach Mts., Anchorage, *Lepage 23404* (CAN, DAO); Chignik, Alaska Peninsula, June 24, July 24 and 25, 1945, *Norberg* (S); King Cove, Alaska Peninsula, *Eyerdam 1504* (S); Moose Pass, *Anderson 6808* (CAN, S); Becharof Lake, *Raup 115* (CAN).

3*f*. SAXIFRAGA PUNCTATA L. ssp. INSULARIS Hult., Sv. Bot. Tidskr. 30: 524. 1936.

Largest leaves 2.9–5.2 cm wide with ca. 18–19 teeth, smooth and somewhat fleshy, glabrous except occasionally ciliate between the teeth; petioles up to 11.5 cm long, entirely glabrous or with a few hairs at junction with blade. Scapes (5.5–)11.0–22.0 cm high, sparingly pubescent below, becoming densely woolly pubescent above, the hairs without evident glands and prominently septate; panicles usually congested, (1.3–)2.5–5.5(–8.8) cm long, 1.4–3.5(–7.0) cm wide, branches stout, usually spreading; pedicels 0.25–0.4 mm in diameter. Sepals 1.3–2.3 mm long, 0.9–1.4 mm wide, green to purplish red; petals (2.5–)3.0–4.5 mm long, 1.2–2.2 mm wide, usually somewhat clawed, nearly always tinged with red; filaments 1.5–2.3(–3.3) mm long, (0.15–)0.2–0.4(–0.5) mm wide, filiform to strap-shaped, elliptic, narrowly triangular or occasionally somewhat clavate, base generally broad, often tinged red; disk often conspicuous in flower, obscure in fruit; stigma 0.3–0.5 mm diameter; capsule 3.0–4.5 mm long, 2.6 mm wide (only a few capsules seen), cleft 15%–30% (–50%) of its length, beaks stout and slightly divergent, nearly always deep purplish red.

*Type*.—Carlisle I., Aleutian Is., *Hultén 6591* (S).

*Range*.—Aleutian Is. and sparingly on St. Paul I. (Fig. 4).

With its thick, shiny leaves, woolly pubescent panicle branches, and generally reddish petals, *S. punctata* ssp. *insularis* is a fairly distinctive plant; but it intergrades smoothly with ssp. *nelsoniana* to the north and ssp. *pacifica* to the east. Some of the intermediate plants cannot be precisely identified.

The collections by Eyerdam from King Cove, Alaska Peninsula, assigned by Hultén (4, p. 929) to var. *krauseana*, are depauperate plants such as one may see in any subspecies of *S. punctata* in wind-swept or otherwise unfavorable situations. They are largely intermediate between *insularis* and *pacifica* but a few are intermediate between *insularis* and *nelsoniana*.

ALASKA: Aleutian Islands: Carlisle Island, *Hultén 6591* (S, type); Agattu Island, *Hultén 6292* (S); Kasatochi Island, *Murcie 2086* (S); Nikolski, Umnak Island, *Hultén 5693* (S); Atka, July 13, 1932, *Hultén* (S), *Hultén 5811* (S), *Hultén 6546* (NY), *Eyerdam 975* (NY), *Eyerdam 1063* (CAN); Adak Island, *Hultén 5910* (S), *Jordal 2962* (CAN); Kanaga Island, *Steenis 4618C* (S); Amlia Island, *Eyerdam 1211* (S); Akutan, *Norberg 365* (S, in part); Unalaska, *Eyerdam 2412* (S); Great Sitkin Island, Andreanof Islands, Aug. 4, 1943, *Kellas & McCarthy* (V). BERING SEA: St. Paul Island, July, 1897, *J. M. Macoun* (CAN).

S. PUNCTATA ssp. INSULARIS – ssp. PACIFICA

ALASKA: Cold Bay, Alaska Peninsula, *Schofield 2423* (DAO); King Cove, *Eyerdam 1512A and B* (S); Olga Bay, Kodiak Island, *E. H. & H. B. Looff 266* (DAO, NY); Karluk, *Horne* (NY); Kodiak, 1910, *Mylroie* (NY); Unalaska, July 25, 1891, *J. M. Macoun* (NY); Chugach Mts., Anchorage, *Dutilly et al. 21171* (DAO).

S. PUNCTATA ssp. INSULARIS – ssp. NELSONIANA

ALASKA: St. Paul Island, Pribilof Islands, *Hultén 7254* (S), Aug. 7, 1891, *Merriam* (NY), 1879, *White* (NY), Aug. 1, 1896, July 1897, June 29, and July 29, 1914, *J. M. Macoun* (CAN); St. George Island, Pribilof Islands, July 13, 1914, *J. M. Macoun* (CAN); King Cove, *Eyerdam 1509* (S), *Eyerdam 1504* (CAN); King Salmon, *Schofield 1982* (DAO); False Pass, Unimak Island, *Eyerdam 1800, 1859, 1875, 1941B, 2003* (S); Shumagin Islands, 1871–1872, *Harrington* (NY); July 21, 1897, *Kinkaid* (S); Akutan, *Norberg 365* (NY, in part); St. Matthew Island, Aug. 10, 1891, *J. M. Macoun* (DAO, CAN, NY); Unalaska, July 4, 1896, and Aug. 2, 1891, *J. M. Macoun* (CAN); *Anderson 4167* (S); Nunivak Island, Aug. 8, 1891, *J. M. Macoun* (CAN); Chignik, *Hultén 775* (S).

3*g*. **Saxifraga punctata** L. ssp. **porsildiana** Calder & Savile ssp. nov.

Laminae foliorum reniformes vel interdum orbiculares, a truncatis valde cordatae, plerumque leviter cordatae, sinu rarissime clauso, majores ca. (1.0–)1.5–3.8 cm lat. dentibus ca. 11–15 mammiformibus vel interdum triangularibus usque ad 0.8 cm long. et 0.9 cm lat. a glabris leniter pubescentes pilis albis vel brunneis; petioli usque ad 5.5 cm long., glabrati. Scapi 8.5–25.0(–28.5) cm alt., ad basas a glabratis leniter pubescentes, ad summas plus minusve lanato-pubescentes, pilis eglandulosis interdum conspicue septatis;

paniculae extensae vel congestae, 1.0–8.5(–12.0) cm long., 1.0–4.5 cm lat., ramis robustis; pedicelli 0.15–0.45 mm diam. Sepala 1.3–2.5(–3.0) mm long., 0.8–1.2(–1.5) mm lat., viridia vel subrufa, saepe glanduliciliolata; petala 2.4–4.6 mm long., 1.0–2.1 mm lat., ab albis rubrotincta, vena maxima saepe conspicua; filamenta 1.5–3.0 mm long., 0.1–0.4 mm lat., a filiformibus ligulata, elliptica vel triangulares, saepe rubrotincta; discus in statu florendi saepe conspicuus, in fructu obscurus; stigmata 0.2–0.5(–0.6) mm diam.; capsulae (3.2–)4.0–7.5(–8.8) mm long., (1.7–)2.0–5.5 mm lat., bifidae ad 40–75%, rostris robustis modice vel valde divaricatis, plerumque purpureo-rubrae.

*Type.*—Murray Range at Azouzetta L., Hart Hwy., B.C. *Calder, Savile, & Ferguson 14079* (DAO).

*Range.*—Interior of Yukon, sparingly in eastern Alaska, northeastward to Bathurst Inlet, eastward to Yathkyed L., and southward to near Banff, Alta. (Fig. 4).

This plant is most appropriately named for Dr. A. E. Porsild, whose studies have contributed materially to the botany of most of the area covered by it; and who, incidentally, collected several of the specimens examined, including the most easterly one on record, from Yathkyed Lake.

*Saxifraga punctata* ssp. *porsildiana* is generally a rather short, stocky plant that seems to be well adapted to exposed arctic and alpine situations. The leaves somewhat resemble those of ssp. *carlottae* in shape, but are less pubescent. In the shape of the filaments and in the generally heavy pigmentation of the flower parts it resembles ssp. *insularis*, but it differs conspicuously from that subspecies in size, shape, and texture of the leaves

Dr. R. J. Moore has determined the chromosome number to be $2n$ = ca. 72 for material from the Itcha Mtns., B.C. An exact count is difficult in this material and the number is possibly 74. Unfortunately no chromosome data are available for any other subspecies of *S. punctata.*

KEEWATIN DISTRICT: Yathkyed Lake, *Porsild 5823, 5824* (CAN); Pelly Lake, *Tener 525* (CAN); Garry Lake, *Corcoran 33* (DAO). MACKENZIE DISTRICT: McTavish Arm, Great Bear Lake, *A. E. & R. T. Porsild 5203* (CAN); junction of Jervoise & Back Rivers, *Tener 296* (CAN); Baillie's River, Aug. 18, 1855, *Rae* (CAN); Contwayto Lake, *Miller 277* (CAN); Bathurst Inlet, *Kelsall & McEwen 245* (CAN); Cape Barrow, Coronation Gulf, *Cox and O'Neill 452* (CAN, NY); Plover and Prairie Lake, Thelon Game Sanctuary, July 28 and Aug. 3, 1936, *Clarke* (CAN); Thelon River, Aug. 12, 1893 and July 10, 1900, *Tyrrell* (CAN); near Yukon border at 62° 30′ N., *Goodwin 4* (NY); Thelon River, Aug. 5, 1951, *Perret & Kelsall* (UBC). YUKON: Mile 10–14, 85–90, 95, and 132, Canol Road, *Porsild & Breitung 9935, 10174, 10175, 10996, 11939* (CAN); Mt. Sheldon, Canol Road, *Porsild & Breitung 11678* (CAN); Canol Road, *M. P. & R. T. Porsild 677* (CAN); Mt. Caribou near Carcross, *Gillett & Mitchell 4585A* (DAO); Smart River, Cassiar Mts., July 28, 1952, *Poole* (DAO); Cassiar Mts., *Poole 106* (DAO); Keno Hill area near Mayo, *Gillett et al. 4261, 4358* (DAO); Mayo, *Broadfoot 28* (DAO); Mayo district, *Bostock 70, 71* (CAN); mountains north of

McQuesten River, *Christie 51* (CAN); Vancouver Creek, McQuesten area, *Campbell 387, 465, 564* (CAN); Kluane Lake, *Clarke 245* (CAN). ALASKA: Cache Mtn., White Mts., *Gjaerevoll 754* (CAN). ALBERTA: Jasper Nat. Pk., *Laing 292* (CAN); summits of Rocky Mts., 52°–56° N., *Drummond* (CAN); Ottertail Pass, Banff Nat. Pk., Aug. 10, 1904, *J. Macoun* (CAN, NY); Lake Louise, July 30, 1904, *J. Macoun* (CAN, NY), *Vanbrunts 58* (NY); Maligne Lake, Jasper Nat. Pk., *Brown 1264* (NY). BRITISH COLUMBIA: near Sheep Creek between 53° and 54° N., *Lambart 67* (CAN); Lake O'Hara, *Sanson 1024* (NY); Murray Range along Hart Highway, *Calder et al. 14079* (DAO, type); Nazcha Camp, Tuya Lake, July 1–5, 1943, *Mathews* (V); Bald Mtn., Glacier Nat. Pk., *Fogg 1239* (UBC); Atlin Lake, *Aitken 34* (DAO); Itcha Mtns., NE. of Anahim Lake, *Calder, Parmelee, & Taylor 20236* (DAO); Maclennan River at headwaters of Fraser River, July 3, 1898, *Spreadborough* (CAN); Cassiar, *Taylor et al. 460, 501, 694* (DAO); Mile 60, 61, 68, and 75 Haines Road, *Taylor et al. 1231, 1202, 911, 827* (DAO).

S. PUNCTATA ssp. PORSILDIANA – ssp. NELSONIANA
BRITISH COLUMBIA: Bennett, *Cowles 992* (DAO).

## Phytogeography

It is clear that *Saxifraga odontoloma* persisted, during the last glaciation, to the south of the Cordilleran ice sheet. Its postglacial northward spread has been slower than that of many plants examined. In the west it has traversed a substantial area in the Cascades of Washington and has reached southern British Columbia, including Vancouver I. In the east it has not penetrated as far into the glaciated region, although the swarm of hybrids with *S. lyallii* make its precise northern limit difficult to define. We have shown (1) that the *Heuchera* complex on the east slope of the Cascades in Washington strongly suggests that glaciation in this area was incomplete, with ridges left projecting between extensive valley glaciers. It is possible that *S. odontoloma* also persisted on these ridges and thus was enabled to traverse the rest of the Cascades promptly as the ice retreated. It is probable that competition from *S. lyallii* partly explains its slow northward spread.

*Saxifraga lyallii* var. *lyallii* appears to have persisted in a refuge on the east slopes of the Montana Rockies, the existence of which we have already indicated (2). It should be noted, however, that the Glacial Map of Canada recently published by the Geological Association of Canada (1958) indicates a small unglaciated area on the east slope of the Alberta Rockies northeast of Crowsnest Pass, which might have provided an additional refuge. It has spread northward along the Rockies to approximately Banff, where it blends with var. *hultenii*. It has probably been driven out of some of its former territory in Montana by climatic changes or competition from *S. odontoloma*. The occurrence of this variety in a narrow strip along the international border west of the Cascades, in country that must have been completely glaciated,

is puzzling. A possible explanation is that the variety spread west from Montana at the close of the Pleistocene but, with its preference for cool and moist conditions, has since disappeared from the intervening area.

We have already noted that *S. lyallii* var. *hultenii* takes different rusts in the northwest and southeast parts of its range, which suggests the existence of two populations; but plants from the whole area seem to be extremely uniform. If var. *hultenii* has spread from an Alaskan refuge throughout the length of British Columbia, its rate of movement has been in excess of what we generally find in plants of similar habitats passing across such highly dissected terrain, such as that of the various subspecies of *S. punctata*. The occurrence of var. *lyallii* in the northwestern Cascades discourages any belief that var. *hultenii* persisted in a Cascades refuge. Our recent discovery of var. *hultenii* in the unglaciated alpine area of the Queen Charlotte Is. suggests that this was one refuge for it and conceivably the only one. Several plants are known to have spread from the Queen Charlotte Is. to the mainland in postglacial times and var. *hultenii* may have been one of them. Its northward and southward spread from a point opposite the islands would have been at a rate approximately that observed in various other plants. Another possibility is that it spread out of separate refuges in the Queen Charlottes and Alaska but that the disjunct populations failed to differentiate perceptibly.

The distribution pattern of *Saxifraga punctata* presents fewer problems than that of the *adontoloma–lyallii* complex. Prior to 1957 we were puzzled by the occurrence in western British Columbia and the Alaska panhandle of plants that we rather dubiously referred to as tending to ssp. *nelsoniana*, because of their pubescent leaves; but such an explanation assumed a southward advance and subsequent retreat of *nelsoniana*. This difficulty was resolved in 1957 by discovery, in the Queen Charlotte Is. refuge, of ssp. *carlottae*, which not only has pubescent leaves but also has other characters of the intergrades not shared by any of the mainland races.

The present distribution of *S. punctata* ssp. *nelsoniana* indicates that its refuge included the northern part of the Bering Sea, much of which was evidently above sea level during the last glaciation. It may have been absent from much of the interior of Alaska; otherwise one might have expected it to have pushed further eastward across Yukon before meeting ssp. *porsildiana*. Ssp. *insularis* occupied a restricted area to the south in the Aleutian Is., bounded by deep water. Its postglacial spread was checked by contact with ssp. *nelsoniana* and ssp. *pacifica* before it could become extensive.

*S. punctata* ssp. *pacifica* presents the most important problem in the North American populations of the species. As we have stated above, it is a valid subspecies with characters that are not intermediate between those of neighboring populations. Yet it is confined to a narrow arc along the coast of the Gulf of Alaska, an area that appears to have been completely glaciated. It is possible that ssp. *pacifica* survived the last glaciation on parts of the continental shelf that are now below sea level. Substantial amounts of the shelf in the Gulf of Alaska must have been above sea level during the Pleistocene

glaciations. In particular, Albatross Bank and Portlock Bank, southeast and northeast respectively of Kodiak I.; the vicinity of Middleton I., south of Prince William Sound; and Fairweather Ground, west of Glacier Bay, should be noted. All these areas are so close to deep water, as well as being appreciably above adjoining parts of the shelf, that they may well have escaped complete glaciation just as the Aleutian Is. did. It could in fact be argued that the very existence of ssp. *pacifica* is evidence that one or more of these areas did escape glaciation.

*S. punctata* ssp. *carlottae* unquestionably persisted on the ice-free upper slopes of the Queen Charlotte Range, whence it spread to the adjacent mainland and inshore islands and merged with ssp. *pacifica* and ssp. *porsildiana*. The Queen Charlotte Is. refuge has been discussed in the preceding two papers of this series. The *carlottae* ancestry of most collections south of Juneau is quite plain, but northwest of Juneau most of the specimens examined are pure ssp. *pacifica*. It is accordingly interesting to note the occurrence of a population from the Prince William Sound region that approaches ssp. *carlottae*. These plants are cited as *pacifica–carlottae* intermediates; but they are closer to *carlottae* than to *pacifica*, and they form a distinctive population in the middle of the range of *pacifica*. It seems clear, from its remoteness from the main range of *carlottae*, that an outpost of *carlottae* must have been established in the Prince William Sound region before the end of the Pleistocene, but whether before the last glaciation or perhaps before the last substage of the latter we have not been able to determine. Possibly it persisted on the bank near Middleton I. mentioned in the preceding paragraph.

*S. punctata* ssp. *cascadensis* persisted either immediately south of the Cordilleran ice sheet or in one or more refuges on the east slopes of the Washington Cascades. The almost complete absence of the plant from Oregon gives some support to the latter alternative. The full extent of the northward spread of ssp. *cascadensis* is somewhat uncertain, for much of the Coast Mts. of British Columbia is highly inaccessible and is still largely unexplored botanically. However it appears that the plant may have once extended further north than it does now, for it is sparingly established on the Queen Charlotte Islands. These Queen Charlotte Islands plants are not quite typical, the leaves being slightly pubescent and often rather shallowly cordate, and the hairs of the panicle being more or less erect rather than appressed. These characters are evidently derived from ssp. *carlottae*, yet the two populations do not generally show complete intergradation, although a few plants from one collection tend about equally to both subspecies. There seems to be a slight ecological difference between the two subspecies, *cascadensis* tending to grow at slightly higher elevations than *carlottae* and to be confined to the edges of runnels rather than being widespread. This difference may partly account for the incomplete blending of the two populations; but the scarcity of *cascadensis*, together with this considerable degree of distinctness, suggests that it reached the Queen Charlottes recently. It may have moved northward along the coast immediately after the Pleistocene or before the last substage of the final

glaciation while the sea level was still sufficiently lowered to facilitate its movement out to the islands against the prevailing wind. The glaciological evidence suggests that the edge of the ice sheet retreated from the coast toward the crest of the Coast Mountains, facilitating coastwise migration. Later climatic changes and the southward movement of ssp. *porsildiana* probably combined to exclude it from the northernmost part of its mainland range.

*Saxifraga punctata* ssp. *porsildiana* presumably persisted through the last glaciation within some part of its present range. The most probable location for such a refuge seemed to be on the eastern flank of the Mackenzie Mts., but until very recently no supporting evidence was available. Flint *et al.* (1949) show extensive unglaciated areas in western Yukon, but little to the east. Dr. H. S. Bostock had indicated to us a considerable eastward extension of this area; but even this extended area was west of the crest of the Mackenzies; and, being connected with the large refuge in the interior of Alaska, parts of which were presumably occupied by ssp. *nelsoniana*, it did not seem probable that another subspecies would persist or differentiate within it. However, as this paper was approaching completion, the Glacial Map of Canada prepared by the Geological Association of Canada appeared. This map indicated one area in the northern Mackenzies and another on the eastern flank of the southern Mackenzies as probably unglaciated. Both of these areas are quite extensive and either might have served as a refuge for ssp. *porsildiana*. The southern one seems the more likely; for *nelsoniana* replaces *porsildiana* near the mouth of the Mackenzie River, an improbable occurrence had *porsildiana* been in the northern Mackenzies; and radiation from a refuge east of the mountains makes the eastward spread of *porsildiana* to Yathkyed Lake comprehensible. It should be noted that *S. punctata* is a plant of moist situations that are generally deeply covered by snow, not of exposed ridges where rapid winter dissemination is likely to occur such as we have postulated for *S. tricuspidata* (2). The limited westward penetration of ssp. *porsildiana* before its replacement by ssp. *nelsoniana* also favors an easterly refuge. It should be noted, however, that there is one record of *porsildiana* in the White Mts., of Alaska, and further collecting may eventually show that it occurs at high elevations along the Ogilvie Mts., which nearly link the Mackenzies with the White Mts.

It may be added that, since the first drafts of this paper were written, one of us (D.B.O.S.) has seen part of the presumptive refugium in the southeast Mackenzies from the air, and that its appearance supports its having escaped glaciation.

The distribution pattern of *Saxifraga punctata* emphasizes the magnitude of the mountain barrier from near Cordova, Alaska, almost to the Skeena River, B.C.; for interchange between coastal and interior plants has been extremely meager over this 700-mile belt of high peaks and nearly continuous snowfields. The general pattern of the species also seems to emphasize the pattern of glaciation throughout northwestern North America.

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# THE EFFECTS OF ETHIONINE ON SOME PLANT GROWTH SYSTEMS[1]

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## Abstract

Ethionine ($CH_3.CH_2.S.CH_2.CH_2.CH.NH_2COOH$) was tested on six plant growth systems: (*a*) growth of lateral buds on decapitated dwarf beans, (*b*) rooting of excised, etiolated bean hypocotyls, (*c*) linear growth and final cell size of primary roots of wheat, (*d*) growth of hypocotyl and roots of flax seedlings, (*e*) growth of sections from etiolated pea shoots, and (*f*) expansion of leaf disks from etiolated beans. Ethionine stimulated growth in each of these systems with the possible exception of (*a*) and (*d*). The work on the rooting of etiolated bean hypocotyls includes the description of a new rooting test.

Pretreatment of pea sections with manganese lowered or prevented the stimulatory effect of ethionine.

Expansion of etiolated bean leaf disks was not increased by ethylenediaminetetraacetic acid, penicillin G, and the 'antiauxins' $\alpha$(2,4-dichlorophenoxy)-isobutyric acid, $\alpha$(*p*-chlorophenoxy)isobutyric acid, and 2,3,5-tri-iodobenzoic acid whereas maleic hydrazide, *o*-chlorophenoxyisobutyric acid, *m*-methyl phenoxyacetic acid, and 2,4,6-trichlorophenoxyacetic acid (TCPAA) stimulated expansion. TCPAA, at 10 mg/liter, completely replaced the stimulatory effect of cobalt on light-treated disks. The peculiar dose–response curve and some morphogenetic effects with TCPAA are described.

Ethionine, cobalt, and TCPAA all inhibit chlorophyll synthesis in leaf disks exposed to light. TCPAA also causes a loss of yellow pigment in disks kept in the dark.

Depending upon the test system, ethionine simulates the effects of auxin or 'antiauxins'. Metabolic studies with ethionine are briefly reviewed. Some connections between the known effects of ethionine and theories of auxin action, and the possible significance of ethionine as an inhibitor of adaptive enzyme synthesis, are pointed out.

## Introduction

Boll (10) reported that ethionine ($CH_3.CH_2.S.CH_2.CH_2.CH.NH_2COOH$), an antimetabolite of methionine, exerts growth stimulatory and morphogenetic effects on excised tomato roots grown in sterile culture. Some of the effects simulated those obtained with either auxins or antiauxins, or those resulting from surgical treatments which interfere with the hormonal, correlative systems in the root (9). It was suggested that ethionine affects the hormonal mechanisms in the root either directly or through an effect upon the metabolism of methionine.

This paper reports experiments to determine the effects of ethionine on some plant growth systems, other than the excised tomato root. Both root and shoot material is included. It was hoped that a marked response to ethionine would be obtained, in a simpler system than the excised tomato root, and thus provide suitable material for critical physiological and biochemical studies of the growth response to ethionine.

Owing to the variety and extent of the growth tests used here, the results have, of necessity, been highly condensed.

[1]Manuscript received February 17, 1960.
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## General Materials and Methods

Etiolated plants used in experiments described here were grown, and maintained after treatment, in a darkroom at a temperature of 24° ± 2° C. The plants were grown in polyethylene dishpans or in stainless steel steam-table trays. The bottoms of the pans and trays were perforated. Seeds were sown in quartz sand (35 mesh; Dominion Corporation Ltd., Lachine, Que.). Before use the sand was washed in tap water and autoclaved in enamel pans for 4 hours at 15 lb pressure. The sterile sand was then partially air-dried. The soaking time of the seed varied with the kind of seed. These times are given in the specific sections below. The pans, except when the plant material was manipulated, were covered with light-tight boxes. All manipulations in the darkroom were carried out in green light. The light source was a Kodak utility safe lamp (model C) containing a 60-w tungsten bulb and a green color filter (Corning Glass No. 4-64, 6 in. × 6 in.).

When etiolated plant material was treated with white light it was placed beneath a bank of eight 48-in. fluorescent lamps (Sylvania 40-w, cool white-standard) giving a light intensity of about 700 ft-c at the plant material. Care was taken to place the plant material within a previously outlined area of uniform light intensity.

Reagent grade chemicals and double distilled water were used for all stock solutions and media. Stock solutions were kept in air-tight vessels and refrigerated at 4° C. Where necessary the pH of media was adjusted with either hydrochloric acid or sodium hydroxide using a glass electrode. The DL-ethionine was purchased from Nutritional Biochemicals Inc.

Statistical analyses were made of the data from some experiments. Comparisons of the differences between means were made by the *t*-test and where necessary, standard errors are included in the tables. Both *t*-tests and standard errors were calculated by the methods given in Snedecor (48).

## Experimental and Specific Materials and Methods

### (A) Lateral Bud Development on Dwarf Green Beans

*Method*

Seedlings of Burpee's Dwarf, stringless, green pod (round pod) bean were grown, three per 5-in. pot, under standard greenhouse conditions. Approximately 23 days after sowing, the internode above the first true leaves measured 2–3 cm and the plants were selected for uniformity. One plant was left in each pot and the selected plants were arranged in three groups according to height. Equal numbers of each of three height classes were included in each treatment. Each treatment contained eight plants.

Plants were decapitated 0.5 cm above the node of the first true leaves. Solutions were applied in small tubes (2–3 ml) as described by Bentley and Bickle (7) except that the tubes were attached with Cello-grease. Solutions were replaced every 24 hours. Each experiment contained four controls: (1) intact, (2) decapitated, (3) decapitated and distilled water placed in the attached tube, and (4) decapitated and a solution of 15 mg per liter 3-indolyl-

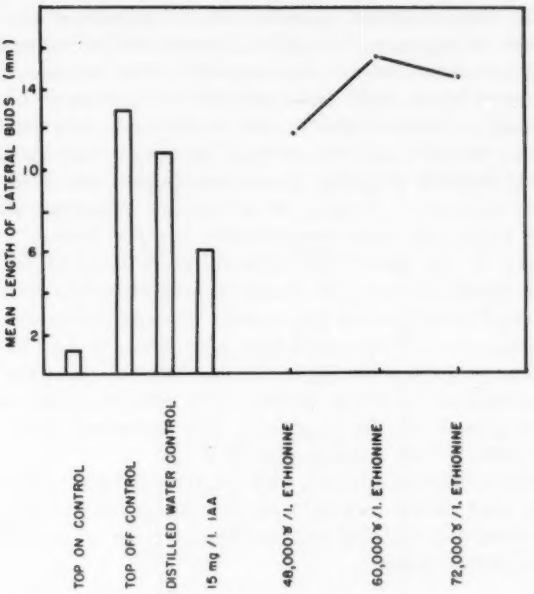


FIG. 1. The effect of ethionine on lateral bud development of decapitated dwarf, green beans.

acetic acid (IAA) placed in the attached tube. The range of concentration of ethionine was from 0.18 to 72,000 gamma per liter. After 7 days the lateral buds were cut off, photographed, and measured.

*Results*

'Catalytic' concentrations of ethionine did not inhibit lateral bud development. However, ethionine at 60,000 gamma per liter, in four out of four experiments, increased the growth of lateral buds over that in the water control. The results of one of the experiments are shown in Fig. 1. Growth in the water control was lower than in the 'top off' control. This was found in all experiments, and was possibly due to a toxic effect of the Cello-grease. This was indicated by a browning of the tissue covered by the Cello-grease. Lanolin, purified according to the method of Redeman, Wittwer, and Sell (40), proved more toxic than either commercial lanolin or Cello-grease. The toxicity was judged following application of the grease either to cut surfaces, or to immature petioles, and observing the browning and distortion of growth.

It is concluded that ethionine promoted the growth of lateral buds but this may, in part, be due to a counteraction of the toxic effects of Cello-grease.

### (B) Rooting of Etiolated Bean Hypocotyls

Attempts to use quantitative rooting tests reported in the literature, and which are suitable for the laboratory, proved unsuccessful. The test described

by Went (65), using etiolated epicotyls of *Pisum sativum* L., was unsatisfactory because of fungal and bacterial contamination in the external sucrose solution. The test described by Hemberg (23), using excised hypocotyls of light-grown, dwarf beans, gave extremely erratic responses to IAA.

In an attempt to devise a rooting test which could be carried out in the laboratory, and which would not require exogenous nutrients, we studied the response of excised, etiolated, dwarf bean hypocotyls to IAA. The IAA promoted root formation. A series of preliminary experiments showed that seedlings over 12 cm high were unsatisfactory and that rooting was decreased in the presence of the apical bud; progressive removal of portions of the cotyledons, in minus IAA controls, caused a progressive decrease in the total number of roots formed, and in the number of roots produced in 10 mg per liter IAA. Amputation of the cotyledons gave little, if any, increase in the rooting response to IAA as measured by the difference between numbers of roots in the control and in 10 mg per liter IAA. With less than one cotyledon, the extension growth of the hypocotyl was decreased and, presumably, endogenous nutrients were limiting growth.

In view of these facts, hypocotyls with the terminal bud and one cotyledon removed were used in the rooting test. The details of the method and the application of this new rooting test to the detection of rooting activity of ethionine is described below.

*Method*

Seeds of Burpee's Dwarf, stringless, green pod (round pod) beans were treated with 2% calcium hypochlorite in water for 15 minutes, rinsed 10 times in distilled water, and then soaked in autoclaved tap water at room temperature for 1.5 hours with one water change every 45 minutes. The seeds were sown about one per square inch on top of a layer of sterile sand which had been soaked with autoclaved tap water and drained for 2 hours. A 1.5-in. layer of partially air-dried sand was placed over the seeds and the pans were placed in the darkroom (see above). All subsequent operations were carried out in green light. After 4 days the seedlings were watered with sterile tap water.

At 5 to 7 days after sowing when most of the hypocotyls were about 10 cm long, the seedlings were harvested. The variation in growing time was presumably because of variations in the temperature and/or water content of the medium. Hypocotyls over 12 cm long were discarded and the remainder were sorted into three groups according to length. These seedlings were trimmed, on a template, to a length of 5 cm as measured from the upper side of the bend in the hook. The epicotyl and one cotyledon were removed. During the trimming operations the seedlings were kept under moist paper towels.

The rooting tests were made in 1.5-oz glasses (F. W. Woolworth). Eight hypocotyls were placed in each glass. This left a liquid capacity of about 45 ml. Two glasses were used in each treatment. All treatments, within one experiment, contained the same number of hypocotyls from each length group. The hypocotyls were arranged so that the terminal hooks clasped the edges of the glass vessels.

Treatment solutions were renewed after 24 hours and the IAA solutions were replaced with distilled water. The liquid in the glasses was then made up to volume daily by adding the appropriate ethionine concentration or distilled water in the case of the IAA treatment. The hypocotyls were examined after 5 or 7 days and the total number of visible roots or primordia were counted on each hypocotyl. Curves showing the dose–response curves for IAA in three successive experiments are shown in Fig. 2. These hypocotyls were examined on the fifth day.

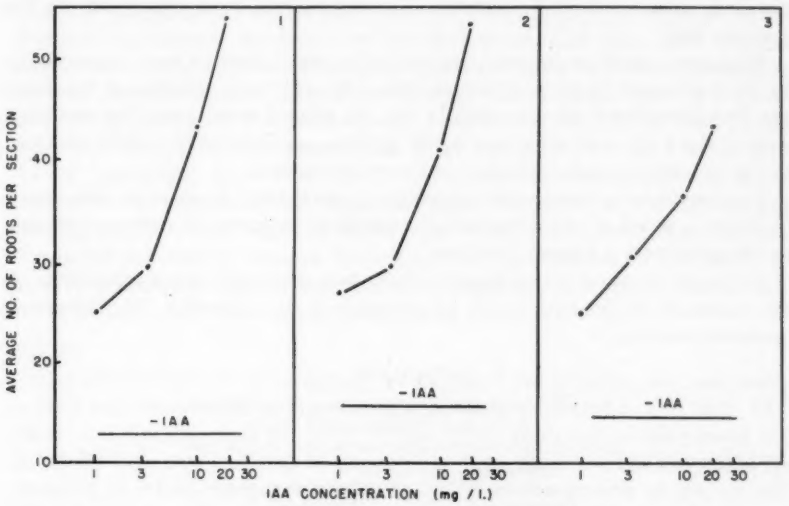


FIG. 2. The effect of concentration of 3-indolylacetic acid on rooting of excised hypocotyls of etiolated green beans. The graphs show three successive experiments.

Subsequent experiments, which cannot be reported in detail here, have shown that there are differences between the rooting responses of the four longitudinal positions in which roots are formed on the hypocotyl. The four positions correspond to the four angles of the hypocotyl which is roughly square in section. If the hypocotyl is held so that it is upright and the terminal hook is bending toward the observer, then the four angles can be identified as front right, back right, front left, and back left. If two cotyledons are present on the section, only the back left and back right positions give a reasonably smooth dose–response curve to the range of IAA concentrations shown in Fig. 2. The front left and front right positions give a hollow curve in which the number of roots is higher with 1 mg per liter than with 3 mg per liter IAA. With one cotyledon (right hand) present the back right position is the only one which gives a consistently smooth dose–response curve and a high response to the higher concentrations of IAA. Thus, if the bean hypocotyl rooting test is used it is recommended that either one (right) or two cotyledons

may be left on but that only the back right row of roots need be counted. This will effect both a saving in labor and a reduction in the variability of the estimate.

*Results: The Effect of Ethionine on Rooting*

The effect of ethionine on rooting was tested with hypocotyl sections from which either the apical bud and one cotyledon or the apical bud and both cotyledons were excised. The range of ethionine concentrations was from 1.8 to 18,000 gamma per liter, and each experiment included appropriate distilled water and IAA controls. The hypocotyls were examined on the seventh day.

Hypocotyl sections bearing one cotyledon were used in two experiments. In the first experiment the seedlings were 5 days old and ethionine at 6 gamma per liter stimulated rooting slightly. In the second experiment the seedlings were 7 days old and ethionine at 60 gamma per liter gave a 27% increase in the number of roots over that in the water control.

The results of a third experiment using decotylized hypocotyls are shown in Table I, which shows a statistically significant increase in root number due to ethionine at 1.8 gamma per liter.

Although in these experiments there was obviously some variation in the response of the hypocotyls to ethionine it is concluded that ethionine promotes rooting.

TABLE I

The effect of ethionine on the rooting of decotylized hypocotyls of etiolated dwarf beans

| Ethionine concentration (gamma/liter) | Number of roots per hypocotyl |
|---|---|
| 0 | 11.7±0.749* (18)† |
| 1.8 | 14.7±0.752 (17) |

NOTE: Probability of the difference <0.01.
*Standard error.
†Number of replicates.

## (C) The Growth and Cell Length of Primary Roots of Wheat

Methods for testing the effects of substances on the growth of seedling roots are listed elsewhere (26, 60). The method used here is a modification of that described by Lexander (29).

*Method*

The seed used in these experiments was *Triticum vulgare* Vill. var. Thatcher obtained from the Department of Agronomy, Macdonald College, Ste. Anne de Bellevue, Que. Seeds were sown endosperm down on filter paper in sterile, 11.0-cm petri dishes. The filter paper was moistened with 8 ml of double distilled water before autoclaving. The seeds were incubated in the dark, at 26° C for 48 hours. After 48 hours, uncontaminated seedlings with straight roots were selected, removed from the dishes, and the primary roots were

measured. Seedlings taken at random were transferred to identifiable positions in 9-cm petri dishes each containing 4.0 ml of treatment solution.

The basal medium was that of Lexander (29). All media were autoclaved for 10 minutes at 15 lb pressure. Each treatment consisted of three dishes with three seedlings in each dish. The seedlings were incubated for 24 hours at 26° C and the primary roots were then measured. The roots were then excised, placed in a fixative (2 distilled water:2 abs. ethyl alcohol:1 glycerol (v/v/v)) and stored until cell lengths were to be measured. About an hour before this time, the roots were pumped in the fixative to remove air bubbles. One root was placed on a slide and covered with a 1:1 mixture (v/v) of 'Javex' (sodium hypochlorite solution) and distilled water. One hour later the root was carefully covered with a cover glass. Care was taken to prevent disarrangement of the epidermal cells by pressure on the cover glass. With an eyepiece micrometer, groups of 10 epidermal cells were measured at 15, 20, and 25 mm from the root tip. Measurements had to be completed within 2 hours after 'Javex' treatment. After this time the cells became disarranged and it was impossible to distinguish the epidermal cells. The effect of the fixative and clearing agent on cell length of wheat roots could not be determined because it was not possible to measure the unfixed and uncleared cells. However, the fixative is known to have little or no effect upon the size of hypodermal cells of the tomato root (Boll, unpublished data).

*Results*

The effect of ethionine on growth in length of the primary root was tested in four experiments using a concentration range of 0.1, 1.0, 5.0, 10.0, 50.0, 100.0, and 500.0 gamma per liter. In two of the experiments the growth was markedly increased at 5 and 10 gamma per liter ethionine. The growth in the presence of ethionine was from 130 to 140% of the growth in the control. Fifty gamma per liter ethionine was supraoptimal. In the other two experiments growth in length was not increased by ethionine and inhibition commenced at 50 to 100 gamma per liter ethionine.

In a fifth experiment the effect of ethionine on the final length of epidermal cells was measured as described above. The values for the control and for roots in 10 gamma per liter ethionine are given in Table II, which shows that ethionine gave a marked increase in the final length of the cells. In view of the effect on cell length, the failure of ethionine to increase the growth in length of the roots in all experiments is puzzling. Although every effort was made to

TABLE II

The effect of ethionine on final length of epidermal cells of wheat roots

| Ethionine concentration (gamma/liter) | Final cell length in eyepiece divisions (1 div'n = 7.0 $\mu$) |
|---|---|
| 0 | 70.4 ± 1.38 |
| 10 | 83.4 ± 1.15 |

NOTE: Probability of the difference <0.01.

keep the conditions constant from one experiment to another it was obvious that when the seedlings were selected they were not always at the same stage of development. It is possible that response to ethionine varied with 'physiological age', and that in some instances any stimulation of increase in cell length by ethionine was offset by an inhibitory effect on cell division. For the purposes of this paper it is sufficient to record that ethionine increases the final size of epidermal cells.

### (D) Growth of Hypocotyls and Roots of Flax Seedlings

Various tests for the effects of growth-regulating substances on seedling growth, particularly of attached radicals, have been described. Most of these are modifications of the method of Swanson (53). The method used here to test the effects of ethionine concentration on the growth of the hypocotyl and radical of flax (*Linum usitatissimum* L.) is a modification of the method of Audus (3).

*Method*

Flax seeds (Dale Laboratories Inc., Montreal) were germinated in the darkroom in a closed cylinder 17 cm in diameter by 25 cm long which was lined with filter paper moistened with autoclaved tap water. After 48 hours the seedlings were selected for uniformity and distributed at random, eight to a treatment chamber. Each chamber was a covered dish 8 cm in diameter and 4 cm deep. Within this, a piece of plate glass 7 cm by 8 cm covered with filter paper rested on the bottom at an angle of 30°. The filter paper dipped into 10 ml of culture solution. Eight seedlings were arranged on the filter paper in a row approximately 4 cm from the base of the glass plate. The basal culture medium contained 1% sucrose and 17 mg per liter calcium sulphate. During the experiment the dishes were kept in a closed glass chamber lined with wet paper towels. The seedlings were measured at 1, 2, and 3 days.

*Results*

Ethionine was tested at 1, 5, 10, 50, 100, 200, 500, 800, 1000, 5000, 10,000, 15,000, and 20,000 gamma per liter. In 11 out of 16 experiments the length of the hypocotyl was increased to a greater or lesser extent by the presence of ethionine (1.0 to 50.0 gamma per liter). Some stimulation of radical growth was observed in only six experiments.

### (E) Pea Section Straight Growth Test

The straight growth test used here was a modification of that described by Thimann and Schneider (58).

*Method*

Seeds of *Pisum sativum* L. var. Alaska were soaked for 4 hours in autoclaved tap water in the dark. They were germinated as described for dwarf green beans except that the peas were covered with one-half inch of dry sand nstead of partially air-dried sand. When the third internode was 1–2 cm long

(after about six days) sections were cut just below the terminal hook with a cutter giving an initial length of about 7.4 mm. Sections were stored in distilled water during the cutting operations and were thoroughly mixed before distribution into the treatment dishes. These dishes were 6-cm petri dishes containing 5 ml of solution and 10 sections per dish. The basal medium was 2% sucrose and the pH was adjusted to 5.5.

Sections were measured against a millimeter ruler under a dissecting microscope. Lengths were estimated to the nearest 0.5 mm by using, in addition to the ruler, a graduated ocular in the microscope.

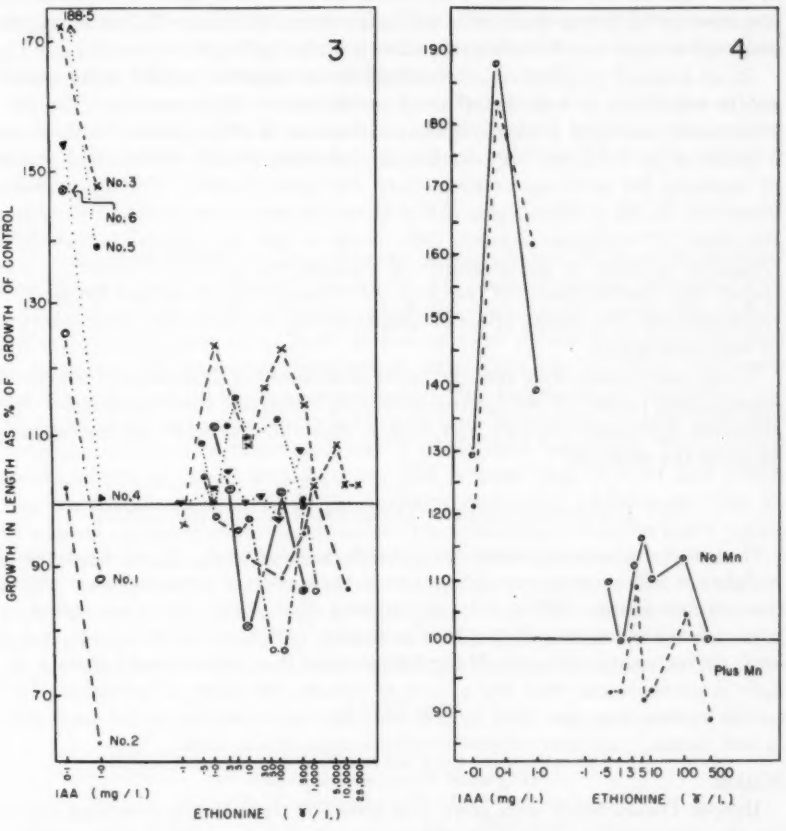


FIG. 3. The effect of concentration of ethionine on the growth of etiolated pea hypocotyl sections in five experiments. The effect of IAA at 0.1 and 1.0 mg per liter is also shown for each experiment except No. 6 in which only 0.1 mg per liter IAA was included.

FIG. 4. The effect of concentration of ethionine on the growth of etiolated pea hypocotyl sections with and without pretreatment with manganese sulphate.

*Results*

Ethionine was tested in a detailed range of concentrations from 0.1 to 25,000 gamma per liter. Controls containing 0.1 and 1.0 mg per liter IAA were included in each experiment and the experiment was repeated four times. Each treatment contained 10 sections with the exception of treatments containing 1, 10, or 100 gamma per liter ethionine. Each of these contained 20 sections. The results are given in Fig. 3, which shows that, from occasion to occasion, there was considerable variation in response to both IAA and ethionine. In spite of this the results are of interest here. In experiment 5 the stimulation of growth at 10 gamma per liter ethionine was significant with a probability of 0.05. If the experiments are compared it will be seen that, generally, when the response to IAA was high then ethionine stimulated growth but when the response to IAA was low then ethionine inhibited growth.

In an attempt to eliminate the variability in response to IAA some experiments were done to test the effect of pretreatment of the sections. The pretreatments included presoaking the sections in distilled water for 1, 2, or 3 hours or in 1 mg per liter manganese sulphate ($MnSO_4.H_2O$) for 3 hours, or exposing the seedlings or sections to red light (Kodak, Wratten, series 1 filter) for 15, 30, or 90 minutes. All of these treatments are believed to reduce the effect of endogenous auxin (38). None of the pretreatments gave any consistent increase in the reliability of the response to IAA. However, it was found that pretreatment of sections with manganese sulphate lowered or prevented the stimulatory effect of ethionine (Fig. 4). This effect was observed in two experiments.

These experiments were not continued because of the difficulties of obtaining reproducible results in the control solutions. However, the results show that ethionine has auxin activity and that it may interact with the endogenous auxin of the sections.

### (F) Expansion of Leaf Disks from Etiolated Beans

If etiolated plants, in which leaf growth is suppressed, are briefly exposed to light the subsequent expansion of the leaves is greatly increased. In studying this photoreaction, Miller (34, 35) showed that disks cut from leaves of etiolated beans expand after a brief exposure to light. This expansion is due solely to increase in cell size. Miller also showed that cobalt would replace the light treatment and that the effects of cobalt and light are additive. This simple system was used here to test the effect of ethionine on cell expansion in leaf tissue.

*Method*

Burpee Dwarf beans were sown and grown in darkness as described above for the hypocotyl rooting test. When the plants were about 31.0 cm high (11 to 14 days after sowing) the first leaves were 1.5 to 2.0 sq. cm in area. The leaves were removed and placed, until all the disks were cut, in a deep petri dish lined with moist filter paper. The disks were cut with a brass cork borer 0.5 cm in diameter. One disk was cut from each side of the midvein

at the base of the leaf. Each disk included a lateral vein approximately at the diameter of the disk. The disks were placed in sterile petri dishes lined with moist filter paper until the cutting operations were completed.

The disks were distributed, upper epidermis down, into 9-cm petri dishes containing two pieces of filter paper moistened with 5 ml of solution. Two dishes, each containing eight disks, were used in each treatment.

The basal medium contained D-glucose (3%) and potassium nitrate (0.08 *M*). Any one experiment had a maximum of four controls. These were (1) basal medium plus green light; (2) basal medium plus white light; (3) basal medium plus cobalt sulphate (5 p.p.m. Cu) plus green light; (4) basal medium plus cobalt sulphate (5 p.p.m. Cu) plus white light. The ethionine concentrations ranged from 0.18 to 18,000 gamma per liter. Media were not autoclaved and pH was adjusted to 5.6. The light source for white light treatments is described above under General Materials and Methods.

Disks were grown for 4 days. They were measured with an ocular micrometer after placing them between two glass slides held together with rubber bands and spaced by binding the ends of one of the slides with adhesive plastic tape.

*Results*

In a series of preliminary experiments, which will not be reported in detail here, it was found that Miller's results (34, 35) could, in general, be reproduced in our laboratory. Disks without white light treatment expanded slightly and a light exposure of as little as one minute caused a marked increase in expansion. With longer exposures to white light the expansion increased slightly and continued to increase up to the longest light treatment (30 minutes). The curve was linear between 1 and 30 minutes. Cobalt increased the expansion both without and with a white light treatment. The optimal concentration of cobalt was 5 p.p.m. The increase due to light and cobalt together was, in contrast to Miller's results, more than additive (cf. Fig. 5). In subsequent experiments reported here the cobalt concentration was 5 p.p.m. and, unless stated otherwise, the exposure to white light was 5 minutes.

The average final diameters of 5-mm disks, as reported by Miller, are given in Table III. These can be compared with the various experiments reported here. In general, the growth of the dark controls was on the order of that reported by Miller. Growth with light treatment, cobalt, or light plus

TABLE III

Mean final diameter of etiolated bean leaf disks, of 5 mm initial diameter, and given the treatments shown, as reported by Miller (35)

| Treatment | Mean final diameter (mm) |
|---|---|
| Dark control | 6.6 |
| Light control | 8.1 |
| Cobalt and darkness | 8.3 |
| Cobalt and light | 9.7 |

cobalt was generally somewhat lower than that obtained by Miller. However, the responses obtained were considered sufficient to justify the use of the test to study the effects of ethionine on disk expansion.

The present experiments are reported in two subsections. The first deals with the effects of ethionine and it is shown that ethionine promotes the expansion of the disks. The second subsection reports the effects of some growth regulators, penicillin G, and ethylenediaminetetraacetic acid which were tested in the hope that their effects might help to explain the stimulation by ethionine.

*(i) The Effect of Ethionine on Expansion of Bean Leaf Disks*

The effect of a range of concentrations of ethionine on the expansion of the disks, with and without light treatment, is shown in Fig. 5. Ethionine at 600 gamma per liter increased the expansion of disks treated with light.

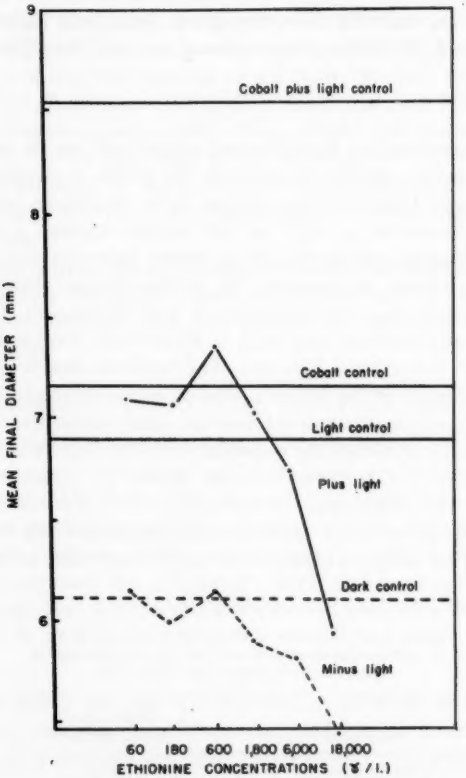


FIG. 5. The effect of concentration of ethionine on the expansion of etiolated bean leaf disks with and without a light treatment. The mean initial diameter of the disks was 4.85 mm.

There was no effect in the absence of light. This result was obtained in a second experiment and in three other experiments ethionine, in a range of concentrations from 0.18 to 60 gamma per liter inclusive, was without effect either with or without the light treatment.

In order to verify the stimulation of growth of the light-treated disks by ethionine at 600 gamma per liter, an experiment was carried out in which large numbers of replicates were included in each treatment. The results are given in Table IV and were left in eyepiece divisions to simplify the statistical calculations. Ethionine again stimulated expansion and the difference between the ethionine treatment and the light control has a probability of less than 0.01.

TABLE IV

The stimulatory effect of ethionine on expansion of etiolated bean leaf disks following exposure to white light. The values are in eyepiece divisions (1 division = 1.88 mm)

| Ethionine concentration (gamma/liter) | Mean final diameter |
|---|---|
| 0 | 3.675 ± 0.0355 (32) |
| 800 | 3.868 ± 0.0505 (36) |

NOTE: Probability of the difference <0.01.

*(ii) The Effects of Ethylenediaminetetraacetic Acid (EDTA), Penicillin G, and Some Growth Regulators on Expansion of Bean Leaf Disks*

EDTA (Bersworth Chemical Co., Framingham, Mass.) was tested at 0.01, 0.03, 0.1, 0.3, 1.0, 3, 10, 30, and 100 mg per liter, on disks given a light treatment but in the absence of cobalt. EDTA was inhibitory at 10 mg per liter and higher concentrations, and no significant stimulation was observed at non-inhibitory concentrations.

Penicillin G (U.S.P. quality, Nutritional Biochemicals Corporation) was tested in two experiments covering a detailed range of concentrations from .01 to 1000 mg per liter. Penicillin G did not stimulate expansion in either experiment and was inhibitory at 300 mg per liter and higher concentrations.

The following is a list of substances which are considered to be antiauxins because they each reversibly counteract the effect of an exogenous auxin in some plant growth system: phenoxyacetic acid or PAA (31), *m*-methyl phenoxyacetic acid or *m*-PAA (1), 2,4,6-trichlorophenoxyacetic acid or TCPAA, *o*-chlorophenoxyisobutyric acid or *o*-CPIBA, α (*p*-chlorophenoxy)-isobutyric acid or *p*-CPIBA, α(2,3,5-tri-iodo)benzoic acid or TIBA, α (2,4-dichlorophenoxy)isobutyric acid or 2,4-DCPIBA (31), maleic hydrazide or MH (26).

Each of these substances was tested on the expansion of leaf disks given a light treatment but no cobalt. The *o*-CPIBA, *p*-CPIBA, 2,4-DCPIBA, and MH were obtained from the California Foundation for Biochemical Research, PAA and TIBA from Eastman Organic Chemicals, *m*-PAA from the Aldrich

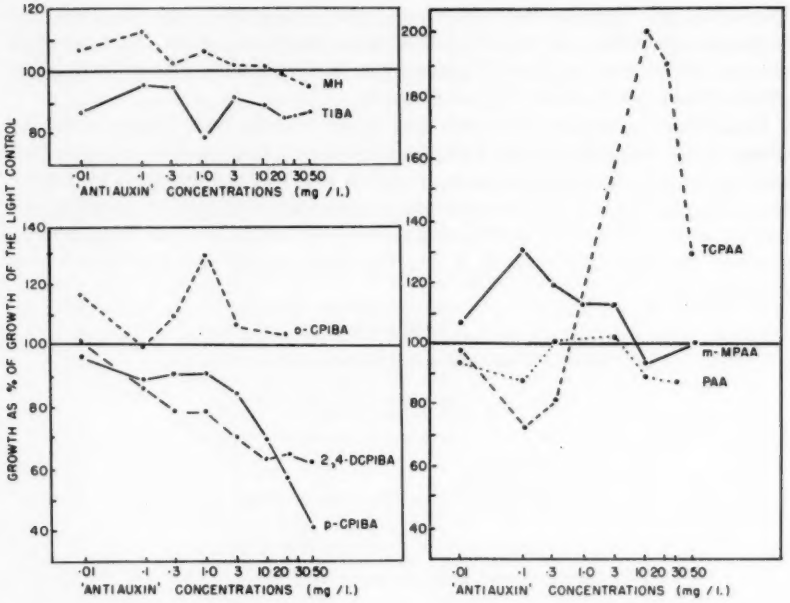


FIG. 6. The effect of concentration of various 'antiauxins' on the expansion of etiolated bean leaf disks. To simplify comparisons the expansion is expressed as a percentage of that in the light control. (For abbreviations see text.) The mean initial diameter of the disks was 4.85 mm.

Chemical Company, and TCPAA from Bios Laboratories Inc. The results are given in Fig. 6. In each of the experiments a dark control and 'light plus cobalt control' were included and showed the typical differences in final diameters. To economize in space they are not included in the graphs.

On the basis of their effects, the compounds fall into three groups. In the first group (2,4-DCPIBA and *p*-CPIBA) inhibition commenced at low concentrations and increased with increase in concentration up to the highest concentration tested. In the second group (MH, *m*-MPAA, and *o*-CPIBA) there was slight stimulation at low concentrations and inhibition at higher concentrations. In the case of *m*-MPAA the inhibition was relieved, to some extent, at the highest concentration. In the third group (TIBA, PAA, and TCPAA) inhibition commenced at low concentrations, was relieved to a greater or lesser extent at higher concentrations, and was inhibitory again at higher concentrations. The most exaggerated form of this unusual curve is in the case of TCPAA with which the inhibition at low concentrations was relieved at higher concentrations in which growth was equal to that in the 'light plus cobalt control'. In other words, at appropriate concentration, TCPAA completely replaced cobalt in the expansion of light-treated leaf disks. Figure 7 shows actual values for the mean final diameter of disks treated with TCPAA.

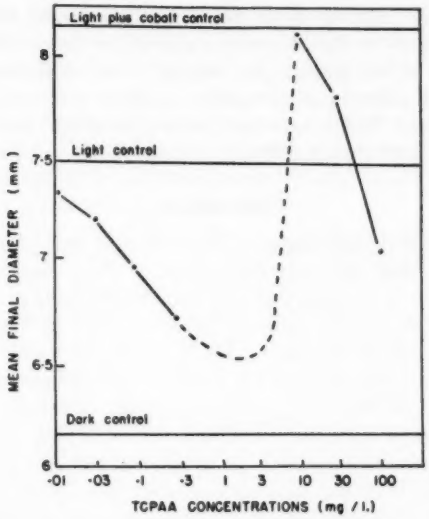


FIG. 7. The effect of trichlorophenoxyacetic acid on the expansion of etiolated bean leaf disks. In concentrations 1.0 and 3.0 mg per liter the disks were too distorted for accurate measurements.

Some interesting incidental observations were made following treatment of the disks with TCPAA. These include the influence of TCPAA on (1) vein, as opposed to intercostal expansion; (2) the retention of yellow pigments during the experiment; and (3) the amount of chlorophyll formed in the disks after an exposure of the disks to 36 hours of light following the TCPAA treatment. After 72 hours in the dark, disks treated with 0.3 to 30.0 mg per liter TCPAA were curled under at the edges. This was particularly marked with 0.3, 1.0, and 3.0 mg per liter. Furthermore, with 3.0 to 30.0 mg per liter the thickness of the veins was considerably greater than in other treatments and the controls. With 100 mg per liter and higher concentrations of TCPAA, growth was inhibited and the edges of the disks were curled but in an upward direction. The disks are placed with the upper epidermis down. Therefore, these observations indicate that either the adaxial as opposed to the abaxial layers of cells, or the veins as opposed to the intercostal areas, respond differently to the antiauxin concentration.

At the time the disks were measured they differed in the amount of yellow pigment present. Disks treated with 0.3 to 100 mg per liter TCPAA were very pale yellow as compared with other treatments and the controls. This suggests that TCPAA either causes a breakdown, or inhibits the synthesis without preventing the turnover, of the yellow pigments. When all the disks in the experiment were exposed to light for 36 hours after they were measured, no chlorophyll formed in the disks which had been treated with 10, 30, or 100 mg per liter TCPAA.

Such inhibition of chlorophyll synthesis was also found when disks treated with either ethionine or cobalt were exposed to light. The inhibition by ethionine occurred at 180 gamma per liter or above. The inhibition by cobalt was evident at 0.5 p.p.m. and complete at about 10.0 p.p.m. cobalt. Thus cobalt, ethionine, and TCPAA, each of which stimulate the expansion of the disks, all inhibit chlorophyll synthesis.

## Discussion

The main purpose of the research reported here was simply to determine whether ethionine could influence the growth of plant systems other than the excised tomato root. A summary of the known effects of ethionine on higher plants, including the results reported here, is given in Table V. The table lists nine growth systems. In two of these (numbers 3 and 4) stimulation of growth would not be expected at the high concentrations of ethionine which were used. Out of the remaining seven systems, ethionine, at relatively low concentrations, stimulated growth in five and possibly all seven.

Ethionine shows auxin activity, as defined elsewhere (61), in that it (i) promotes the growth of sections of etiolated pea epicotyls, (ii) promotes rooting of bean hypocotyls, and (iii) increases the production of lateral roots on excised tomato roots (10). This latter effect is produced by NAA (9) and

TABLE V

Summary of known effects of ethionine on the growth of higher plants

| Test system | Stimulatory concentration (gamma/liter) | Inhibitory concentration (gamma/liter) | Reference |
|---|---|---|---|
| 1. Pea epicotyl sections, straight growth test | 10 | >25,000 | Reported here |
| 2. Expansion of etiolated bean leaf disks | 600–800 | 1,800 | Reported here |
| 3. *Avena* coleoptile sections, straight growth test | Low concn. not tested | 10,000 (50% inhibition) | Schrank (41) |
| 4. *Avena* coleoptile, curvature | Low concn. not tested | 2,000 | Schrank (41) |
| 5. Dwarf bean, lateral bud growth | (?) 60,000 | >72,000 | Reported here |
| 6. Etiolated flax seedlings: growth of hypocotyl | (?) 1–50 | 500 | Reported here |
| 7. Etiolated dwarf bean: rooting of excised hypocotyl | | | |
| (i) plus one cotyledon | 60 | 300 | Reported here |
| (ii) decotylized | 1.8 | 300 | Reported here |
| 8. Wheat seedlings: growth and final cell size in primary root | 10 | 100 | Reported here |
| 9. Excised tomato roots: | | | |
| (i) minus niacin medium | | | Boll (10) |
| Growth of main axis | 6.0 | 12.0 | |
| No. of laterals | 6.0 | 30.0 | |
| Growth of laterals | 30.0 | 60.0 | |
| (ii) plus niacin medium | | | |
| Growth of main axis | 12.0 | 30.0 | |
| No. of laterals | 12.0 | 30.0 | |
| Growth of laterals | 1.2 | 60.0 | |

IAN (52). On the other hand ethionine also stimulates the growth of systems which are not known to be stimulated by auxins but which are stimulated by 'antiauxins'. Thus Burstrom, Hansen, and Hansen *et al.* (see ref. 2) reported that various 'antiauxins' increase the final size of cells in wheat roots. Street (50, 51) and Boll (9) have shown that 'antiauxins' increase the growth in length of excised tomato roots and, in this paper, it is shown that certain 'antiauxins', particularly trichlorophenoxyacetic acid (TCPAA), increase the expansion of etiolated bean leaf disks.

Thus, although the stimulatory effects of ethionine on the growth of plant cells is not simply because it acts as an auxin, it is, nevertheless, reasonable to conclude that ethionine interferes with the action of native growth regulators. Our present knowledge of the metabolic effects of ethionine gives some, although very few, clues as to the way in which such an interference might result. This is seen from the following brief review. To help clarify the discussion the metabolic pathways from methionine are illustrated in Fig. 8.

Ethionine may interfere with transmethylation in that the ethyl group of ethionine is transferred to compounds which normally receive the methyl group from methionine. One such example is the formation of triethylcholine in rats fed ethionine (30). The lipotropic action of ethionine in rats (17, 18, 19, 24) is considered to be due to interference with transmethylation in the biosynthesis of choline and creatine (44). On the other hand transethylation does not occur in some biological systems (16), which suggests that under some conditions, the entire ethionine molecule may prevent transmethylation.

In transmethylation, methionine first reacts with ATP to form an adenine sulphonium compound S-adenosylmethionine (12). Schlenk and Tillotson (42) showed that 5-ethylthioadenosine was synthesized by yeast in ethionine-enriched cultures and Axelrod (4) reported that ethionine reacts with ATP to give ethylthioadenosine. Recently, Parks (39) showed that ethionine leads to the biosynthesis of S-adenosylethionine in yeast. Transethylation between

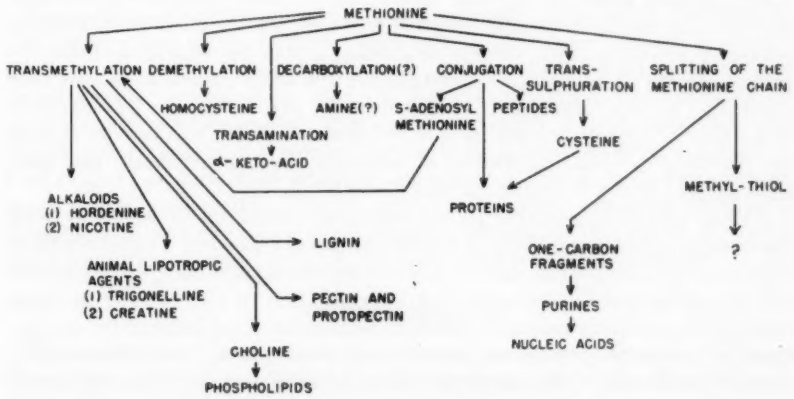


FIG. 8. Metabolic pathways from methionine.

this substance and homocysteine occurred in vitro. Ethionine suppressed the formation of S-adenosylmethionine and there was some evidence for competitive interference between the ethyl and methyl donors. Presumably the S′-adenosylethionine leads to a variety of abnormal ethyl derivatives and thence to a failure of normal processes in cells.

Ethionine is not known to inhibit the biosynthesis of homocysteine from methionine. In fact, Stekol and Weiss (49) showed that ethionine labelled with $C^{14}$ in the methyl C of the ethyl group, and with $S^{35}$, was available for cysteine synthesis. This suggests that ethionine is available for homocysteine synthesis. On the other hand Farber *et al.* (17) found that ethionine inhibits cysteine synthesis in rats.

Ethionine does not inhibit transamination from methionine but undergoes transamination itself (32, 33). Presumably the $\alpha$-keto-acid derivative of ethionine is formed and this may be an inhibitory analogue of the corresponding methionine derivative. This has not been shown experimentally.

There is no evidence to suggest that ethionine interferes with the splitting of the methionine molecule in the formation of methylthiol. However, Smith (47) reported that ethionine was shown, by Swenseid and co-workers, to prevent the enzymatic formation of one-carbon fragments in rats. According to Smith the actual inhibitor is a substance produced enzymatically from ethionine. One-carbon units from methionine are necessary for nucleic acid synthesis. This suggests a point at which ethionine might disturb the metabolism of nucleic acids.

Thimann and Radner (56, 57) reported that both ethionine and methionine inhibit anthocyanin synthesis in *Lemna minor* L. One possible explanation of this fact is that the inhibition may be due to some part which is common to both the ethionine and methionine molecules. In other words, inhibition by ethionine, in some cases, may be due to the conversion of ethionine to a product which is normally formed from methionine.

Experiments either using tracer techniques, or which studied the effects of ethionine on the formation of adaptive enzymes, show that in some systems ethionine interferes with protein synthesis. Ethionine interferes either by preventing the incorporation of methionine and other amino acids and thus preventing protein synthesis (17, 45, 66), or by preventing the incorporation of methionine by substituting for it in the protein (22, 28) and thus producing an abnormal protein. Inhibition of adaptive enzyme synthesis has been reported by Lee and Williams (25) and Barrett *et al.* (5).

All the above information on the metabolic effects of ethionine is from experiments involving inhibitory concentrations of ethionine. The information may, therefore, be of little value in any attempt to explain the growth stimulation by ethionine. Furthermore, the interpretation of effects such as those reported here is made especially difficult because our knowledge of the mechanism of action of growth regulators is so inadequate. Nevertheless some connections between the metabolic effects of ethionine and theories of the mechanism of auxin action are worthy of note.

Explanations of auxin action have postulated, amongst other suggestions, (i) a chelating action (6); (ii) control of nucleic acid synthesis and ratios of RNA to DNA (6, 46); (iii) a stimulation of pectin methyl esterase activity by auxin thus giving demethylated chains which, compared with methylated chains, are destroyed more readily by galacturonase; this leads to a plasticizing and growth of the wall (13, 37, 64); (iv) a combination of auxin with sulphydryl groups on some protein which is possibly an enzyme involved in the 'growth reaction' (see refs. 11, 26, 27, 36, 43, 54, 55, 59, 62, 63). It is shown here that EDTA does not stimulate the expansion of etiolated bean leaf disks. This suggests that, at least in this system, the stimulation by ethionine was not due to chelating properties. However, in the case of each of the other explanations of auxin action listed above, there are links with known metabolic effects of ethionine (see above).

Some aspects justify further comment. Thus it is shown here that penicillin G and TIBA, substances which are believed to interfere with growth by combining with sulphydryl groups (14, 15, 27), did not reproduce the effects of ethionine on the expansion of etiolated bean leaf disks. Furthermore, stimulation of cell growth by cobalt is considered to be due to a combination of cobalt with sulphydryl groups (55), but whereas cobalt stimulates expansion of etiolated bean leaf disks both with and without a light treatment, ethionine acts only following a light treatment. On this evidence it seems unlikely that stimulation by ethionine, at least in the leaf disk, is due to an effect upon sulphydryl metabolism or groups.

There are no indications from the work presented here that growth stimulation by ethionine is due to the formation of an ethyl analogue of some normal metabolite following transethylation from ethionine. However, this possibility cannot be neglected because it has been shown elsewhere that the morphogenetic effects of 2-diethylaminoethanol (a synthetic analogue of 2-dimethyl ethanol) on the excised tomato root (8) resemble those of the 'antiauxin' 2,4-dichloroanisole (9).

In concluding this discussion one further point should be noted. The enzyme IAA oxidase may be an adaptive enzyme (21) and Galston (20) has developed the view that this characteristic may explain many developmental and morphogenetic relationships of IAA. Ethionine inhibits the synthesis of adaptive enzymes (see above). It is therefore possible that the auxin-like and morphogenetic effects of ethionine are due to an inhibition of the synthesis of IAA oxidase, or some other adaptive auxin oxidase, which raises the effective level of endogenous auxin. If this suggestion is correct, ethionine could be a valuable tool in physiological experiments in which it is desirable to eliminate or stabilize the enzymic destruction of auxin. This is being investigated.

## Acknowledgments

This work was made possible by financial assistance from the National Research Council of Canada. The figures were prepared with the able assistance of Mrs. Norah J. Nolan.

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